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### CORRIGENDA IN SUPPLEMENTARY VOLUME

- p. 159, col. 2, l. 9, for affect read effect.
- p. 159, col. 2, l. 29, for Nigeria read Victoria.
- p. 159, Table 1, last line, for Early (enozoic) read Early Cenozoic.
- p. 161, Table 2, for *Hastert's* read *Hartert's*.
- p. 162, col. 1, l. 26, for depend read depends.
- p. 162, col. 2, l. 48, for *Vira* read *Uria*.
- p. 163, col. 2, l. 30, for interpluvian read interpluvial.
- p. 164, under References: HUXLEY, J. S. (1938a) for de Bear read de Beer.

## THE GENETICS OF COTTON

XVIII. TRANSFERENCE OF GENES FROM DIPLOID NORTH AMERICAN WILD COTTONS (*GOSSYPIUM THURBERI*, TOD., *G. ARMOURIANUM* KEARNEY, AND *G. ARIDUM* COMB.NOV. SKOVSTED) TO TETRAPLOID NEW WORLD COTTONS (*G. BARBADENSE* L. AND *G. HIRSUTUM* L.)By SYDNEY CROSS HARLAND AND  
OLGA M. ATTECK

(With Plates 1 and 2)

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## INTRODUCTION

In a previous paper (Harland, 1935a) it was shown that it was possible to obtain a hybrid between *Gossypium arboreum* L., the cultivated Asiatic cotton with  $n=13$  chromosomes, and *G. barbadense* L., the cultivated American cotton with  $n=26$  chromosomes. Although the  $F_1$  of this cross was completely sterile on the female side, it manifested slight fertility on the male side. This rendered it possible to backcross the  $F_1$  on to *G. barbadense* L. and later on to *G. hirsutum* L. After several backcrosses complete fertility was restored. The object of the experiment was to transfer by repeated backcrossing the gene **R** (red plant body, red flower, petal spot) from the *arboreum* parent to a *hirsutum* background, (green plant body, cream flower, no petal spot), and then to investigate the linkage relations of the transferred gene to the anthocyanin locus containing, among other genes, **S<sup>H</sup>** (*hirsutum* petal spot). These experiments demonstrated that the Asiatic gene **R** was a member of the New World multiple allele series for anthocyanin pigmentation, and this confirmed on genetical grounds the theory of Skovsted (1934a) that the New World  $n=26$  group is amphidiploid with one subgenom homologous with the  $n=13$  Asiatic species *G. arboreum* and *G. herbaceum*.

Cytological studies by Skovsted (1934a) and Webber (1934, 1935, 1936) led these authors to the belief that the other subgenom of the New World  $n=26$  group was derived from a type probably ancestral to the present North American diploid wild cottons, comprising the four species *G. Thurberi* Tod.,<sup>1</sup> *G. Armourianum* Kear., *G. aridum* comb. nov. Skov., and *G. Harknessii* Brandg. That these four diploid species are closely related is evident, both from cytological studies and crossing relationships (Skovsted, 1937; Webber, 1935, 1936), since the crosses *Thurberi*  $\times$  *Armourianum*, *Thurberi*  $\times$  *aridum*, and *Armourianum*  $\times$  *Harknessii* are reasonably interfertile. The fact that crosses of these diploid species with tetraploid *barbadense* were shown not only to be possible, but also to exhibit a slight degree of fertility on the male side, rendered it likely that genes from these species could be transferred to the background of *hirsutum* or *barbadense* and the linkage relations of such transferred genes studied in their new genetical environment.

Harland (1937b) gave a preliminary account of certain successful transferences, and this paper comprises a more extended account of the transference of certain genes from *G. Thurberi*, *G. Armourianum*, and

<sup>1</sup> Formerly known as *G. trilobum* Moq. & Sess. ex DC. but assigned to *G. Thurberi* Tod. by Kearney (1937).

*G. aridum* by means of repeated backcrosses to the genetical background of *G. hirsutum* or *barbadense* and of their linkage relationship to known New World genes when so transferred.

#### MATERIAL

*G. Thurberi*, *G. Armourianum*, and *G. aridum* were each crossed with *G. barbadense* L. (Sea Island Crinkled Dwarf). The  $F_1$  was made without any difficulty and a considerable number of plants obtained from each cross. The fertility of the crosses was as follows:

*barbadense*  $\times$  *Thurberi*: weakly fertile female and male (Harland & Atteck, 1931).

*barbadense*  $\times$  *Armourianum*: partly fertile male, sterile female (Skovsted, 1935).

*barbadense*  $\times$  *aridum*: partly fertile male, sterile female (Skovsted, 1935).

To obtain the first backcross to *barbadense* was difficult, and a large number of attempts were necessary before success was obtained. Several hundred pollinations were made using  $F_1$ 's as females, but the bolls all shed after a few days. Plants of the first backcrosses were considerably more fertile and no difficulty was experienced in making further backcrosses.

#### CHARACTERS TRANSFERRED

Below are presented data on the characters transferred from the diploid to the tetraploid:

Species	Character	Character in <i>barbadense</i>
<i>G. Thurberi</i>	Normal	Crinkled
<i>G. Armourianum</i>	Normal	Crinkled
	Large petal spot	Smaller petal spot
	Tinged calyx	No anthocyanin
	Tinged flower	No anthocyanin
<i>G. aridum</i>	Normal	Crinkled
	Large petal spot	Smaller petal spot
	Purple corolla	No anthocyanin
	Tinged calyx	No anthocyanin

#### NOTES ON CHARACTERS

*Normal-crinkled*. It has been established (Harland, 1916, 1918) that the crinkled mutant of *barbadense* (Sea Island) is recessive to the normal type with normal completely dominant. In order to see whether the three wild species possessed a normal allele of the crinkled mutant, the Sea Island variety Crinkled Dwarf was used as a base. The grades of

crinkled used in classifying the material are those previously described (Harland, 1935b). Grade 7 is normal, 6.5 slightly crinkled, 6 distinctly crinkled, 5 strongly crinkled, and 4 is the crinkled *barbadense* standard.

*Petal spot.* *Armourianum* has a larger and more intense petal spot than *barbadense* (var. Sea Island). The outer edges of the petals are tinged with anthocyanin and also the calyx.

*Aridum* has a deep purple corolla and a petal spot which occupies almost the whole of the lower third of the petal. The calyx, column, and filaments are also strongly pigmented.

## THE EXPERIMENTS

### A. EXPERIMENTS WITH *G. THURBERI* TOD.

#### (1) *Transference of normal (Thurberi) to crinkled (barbadense)*

*The F<sub>1</sub>.* The *F<sub>1</sub>* plants were uniformly though only slightly crinkled (grade 6.5).

*The first backcross.* Only one plant was obtained, slightly crinkled, of grade 6.

*The second backcross.* Fifty-one plants were grown and these were clearly divisible into two groups, typical short *barbadense* crinkled and tall intermediates, mostly of grades 5.5, 6, and 6.5, with considerable variation in the expression of crinkled. The numbers were:

Intermediate: Heterozygotes	21
Crinkled	30

*The second backcross selfed.* As all material of the third backcross was lost when the writer transferred his experiments from Trinidad to Brazil it was only possible to continue the experiment with a selfed culture of one of the intermediate heterozygotes. Plant C 2065 segregated into:

	Normal	Intermediates	Crinkled
	4	21	8
Expected 1 : 2 : 1	8.25	16.5	8.25

Most of the plants were fully fertile and near Sea Island *barbadense* in appearance. One of the normals, C 2893, was selfed and produced sixteen normal plants in the next generation.

*The third backcross.* Another normal plant (C 2913) was crossed with *barbadense* crinkled and gave nine plants all intermediates of grade 5.5-6. All the plants were fully fertile, with no sign of sterility.

The fourth backcross. Two families were grown with the following results:

Family	Intermediates grades 6 and 6.5	Crinkled
C 5078 × 4-C 5548	57	57
C 5080 × 4-C 5548	49	49
Total	106	106
Expected 1 : 1	106	106

To sum up: these experiments show clearly that *G. Thurberi* Tod. (= *Thurberi thespesioides* A. Gray), the diploid wild cotton of Mexico and Arizona, contains a normal allele of the crinkled gene of the tetraploid cultivated *G. barbadense* L. (Sea Island variety). The normal allele of *Thurberi*, however, differs in dominance potency from the corresponding normal allele of *barbadense* in that it fails to produce complete dominance in the heterozygote. In this respect it resembles the normal alleles of crinkled found in Upland (*G. hirsutum*), Bourbon (*G. purpurascens*), and *G. Taitense* Parl. (Harland, 1936; Harland & Atteck, 1940).

#### B. EXPERIMENTS WITH *G. ARMOURIANUM* KEARNEY

Crosses were easily made with both *barbadense* and *hirsutum*. The hybrid with *barbadense* (Sea Island) was slightly fertile on the male side and sterile on the female side, while the hybrid with *hirsutum* (Upland) was completely sterile on both male and female sides. Thus the transference of a character from *Armourianum* to *hirsutum* can only be done by crossing with *barbadense* twice and then crossing the resultant material which possesses the desired *Armourianum* characters and restored fertility, to *hirsutum*.

Two more generations of crossing with *hirsutum* are then necessary to convert the material into types which, although predominantly *hirsutum* in morphological characters, carry considerable numbers of genes from both *barbadense* and *Armourianum*.

##### (1) Transference of normal (*Armourianum*) to crinkled (*barbadense* and *hirsutum*)

The  $F_1$ . *G. barbadense* (crinkled) × *G. Armourianum* (Kearney). Of seven plants, six were normal (grade 7) and one plant slightly crinkled (grade 6.5).

The first backcross (× *barbadense* crinkled). Only one plant was obtained after more than 100 pollinations. This plant (G 9771) was normal (grade 7) and fairly fertile both on male and female sides.



*The first backcross selfed.* The second backcross cultures were lost and it was only possible to continue the experiment by using plants from the first backcross selfed. The results were:

		Heterozygotes	
Crinkled	Grade 6	6.5	7
3	2	—	10
		12	

The ratio here obtained is not far from 3:1, and dominance of normal is nearly complete. A single normal (C 439) was selected to backcross to various types of crinkled.

*The second backcross.*

	Heterozygotes	Crinkled
(a) C 439 × <i>hirsutum</i> type 9 crinkled	29	18
Expected	23.5	23.5
(b) C 439 × <i>barbadense</i> type 4 crinkled	18	15
Expected	16.5	16.5

In both series of crosses the normals were completely devoid of any trace of crinkling, with the exception of one plant of grade 6.5.

*G. Armourianum* Kearney thus has a normal allele of crinkled which resembles the normal allele of *barbadense* in being completely dominant on the *barbadense* background, whereas it will be recollected that the normal allele of *G. Thurberi* Tod. was not completely dominant. Further backcrossing to *hirsutum* type 9 crinkled will be necessary to determine whether the normal allele of *Armourianum* is also completely dominant on this background.

(2) *Transference of petal spot (Armourianum) to barbadense and hirsutum*

*G. Armourianum* generally has a large and vivid spot at the base of the petal, although a recessive spotless form has been recorded (Harland, 1937c). Anthocyanin is also present in the filaments and anther cases, and along the upper edges of the calyx.

The type *G. barbadense* (Sea Island) used in these experiments has a spot of grade 13 (classification of the writer, Harland, 1929), but this is less intense than the *Armourianum* spot.

*The F<sub>1</sub>.* Grade of spot 20 with strongly pigmented filaments.

*The first backcross.* Two plants were obtained. One possessed a large and intense spot of grade 18 with red filaments and the other was practically spotless.

*The first backcross selfed.* The progeny of the plant with *Armourianum* spot segregated into four strongly spotted of *Armourianum* type with coloured filaments and two weaker spotted of *barbadense* type.

*The second backcross ex first backcross selfed (to hirsutum).* One plant, C 437, from the first backcross selfed, with *Armourianum* spot and coloured filaments, was crossed with a red *hirsutum* ( $R^H$ ) for two reasons: first, to continue the transference of *Armourianum* spot (henceforth denoted  $S^{ARM}$ ) to the *hirsutum* background, and second to determine the linkage relationship of  $S^{ARM}$  to  $R^H$ .

A family of seven plants was obtained, all with typical *Armourianum* spot. The parent was evidently homozygous for spot.

*The third backcross (to hirsutum).* Linkage relationship of  $S^{ARM}$  and  $R^H$ . The seven plants heterozygous for  $S^{ARM}$  and  $R^H$  were backcrossed to double recessive *hirsutums* and the following results obtained:

Table 1. Results of backcrosses of heterozygotes  $R^H S^{ARM} r^H s^{ARM}$  to recessive *hirsutum*

Heterozygous parent ...	$R^H S^{ARM}$	$R^H s^{ARM}$	$r^H S^{ARM}$	$r^H s^{ARM}$
1980	0	140	155	0

(a) Genetic relationship of  $S^{ARM}$  and  $R^H$  (*hirsutum* red)

The plants in this, the third backcross, were all completely fertile and segregation into  $S^{ARM}$  and  $s^{ARM}$  took place in the typical 1:1 ratio. The spot was large and intense and was accompanied by red filaments, spot and filament colour being apparently inseparable. Further, there was complete repulsion between  $S^{ARM}$  and  $R^H$ . Not only, therefore, is it possible to transfer the spot gene  $S^{ARM}$  from *Armourianum* to *hirsutum* but that when so transferred it is found to be a new member of a multiple allelomorphic series, formerly known to contain only two members  $R^H$  (*hirsutum* red) and  $r^H$  (*hirsutum* green). It should be especially noted that  $S^{ARM}$  is not known in New World tetraploids; it is a new gene, and in this respect it resembles  $R^A$  (Asiatic red), which when transferred from *arboreum* (Asiatic diploid) to *hirsutum* (New World tetraploid) was also a new member of an anthocyanin multiple allele series which included  $R^B$  (*barbadense* red),  $S^H$  (*hirsutum* spot) and  $S^B$  (*barbadense* spot). Now *hirsutum* red  $R^H$  and *barbadense* red  $R^B$  have long been known to be a pair of independently inherited duplicate genes, and it is now possible to state that they are respectively situated in the two constituent Asiatic and North American 13-chromosome genomes. Other pairs of duplicate genes are known to exist in New World cottons, and it is now more than

a conjecture that members of these pairs will presently be assignable to Asiatic and New World genoms respectively. Finally, it should be noted that  $S^{ARM}$  on a *barbadense* background was extremely large and intense. On a *hirsutum* background it was also powerfully manifested.

### C. EXPERIMENTS WITH *G. ARIDUM* (COMB. NOV. SKOV.)

#### (1) Transference of normal (aridum) to crinkled (barbadense)

*The F<sub>1</sub>*. Plants were uniformly slightly crinkled of grade 6-5.

*The first backcross*. Four plants were obtained in the heterozygous group. One was normal (grade 7) and the rest slightly crinkled. Some crinkled plants also appeared but the records were lost.

*The second backcross*. Two families were grown. The first family gave five typical crinkled of grade similar to *barbadense* type 4, and six heterozygotes of grades 5-5-6-5 with much variation. One plant, C 331, was first recorded as a normal but when old became slightly crinkled. The other family gave two crinkled and two heterozygotes of grade 6-5.

*The third backcross*. In the third backcross the heterozygote was crossed with *hirsutum* crinkled of two types.

Results of third backcross of heterozygote with *hirsutum* crinkled type 252:

Family	Grade of crinkled					
	3	4	5	6	6-5	7
C 331 × type 252	6	6	—	3	5	3
	12			11		
	crinkled			heterozygotes		

Here we have 12 crinkled : 11 heterozygotes, three of which were phenotypically normal.

Results of third backcross of heterozygote with type 57 *hirsutum* crinkled:

Family	Grade of crinkled					
	3	4	5	6	6-5	7
C 331 × type 57 crinkled	2	5	—	2	1	2
	7			5		
	crinkled			heterozygotes		

Here there were again two distinct groups, a typical crinkled group (grades 3 and 4) and a heterozygous nearer normal group (grades 6-7), with approximately a 1 : 1 ratio.

*The fourth backcross.* Some plants of the third backcross to type 57 crinkled were again backcrossed with type 57 crinkled and gave results as follows:

Family	Grade of crinkled					
	3	4	5	6	6.5	7
G 9-24 × type 57 crinkled	7	1	—	—	6	4
G 9-28 × type 57 crinkled	—	—	—	—	1	2
G 10-34 × type 57 crinkled	18	8	—	—	22	6
Total	25	9			29	12
	34			41		
Expected 1 : 1	37.5			37.5		

To sum up: It is demonstrated that *G. aridum*, as well as *G. Thurberi* and *G. Armourianum*, possesses a normal allele of the crinkled mutant of *barbadense*. So far it appears that the dominance potency of this allele is not so great as that of *Armourianum* or *barbadense*.

## (2) Transference of petal spot (aridum) to barbadense

*G. aridum* has a small purple flower with a large petal spot occupying almost the whole of the lower half of the petal. The filaments, anther cases, and calyx are also strongly pigmented. The object of the experiment was to see whether this anthocyanin complex is inherited as a single-unit character when backcrossed to tetraploids. It should be noted that the anthocyanin complex of *aridum* differs principally from that of *Armourianum* (a) in the strongly pigmented petal, and (b) in the stronger and more intense petal spot. The whole colour complex will be denoted as **S<sup>ARI</sup>**, and its absence as **s<sup>ARI</sup>**.

*The F<sub>1</sub>* (*barbadense* × *aridum*). Grade of spot was much higher than grade 22, the maximum grade previously recorded in any experiment concerning petal spot, being approximately grade 25.

*The first backcross.* Several plants were obtained, but only two (G 10315 and G 9533) showed the *aridum* anthocyanin complex. Coloration was strongly reduced. The corolla was moderately flushed purple; spot was about grade 20 and filaments strongly coloured.

*The second backcross.* The two plants of the first backcross which possessed the *aridum* anthocyanin complex were crossed with *barbadense* and gave the following results:

Family	S <sup>ARI</sup>	s <sup>ARI</sup>
<i>barbadense</i> type 4 × G 9533	6	4
<i>barbadense</i> type 4 × G 10315	5	18
Total	11	22
Expected 1 : 1	16.5	16.5

Here there is a deficiency of **S<sup>ARI</sup>** plants. Some sterility (male and female) was still present in many plants.

*The third backcross.* Four plants of the third backcross with the *aridum* anthocyanin complex, **S<sup>ARI</sup>**, were crossed either with *barbadense* or with *hirsutum* recessives, giving results as follows:

Family	<b>S<sup>ARI</sup></b>	<b>s<sup>ARI</sup></b>
<i>hirsutum</i> × C 331	133	141
<i>barbadense</i> × C 329	2	3
<i>hirsutum</i> × C 329	78	105
<i>hirsutum</i> × C 335	5	3
<i>barbadense</i> × C 335	2	2
<i>barbadense</i> × C 1514	10	17
<i>hirsutum</i> × C 1514	121	145
Total	351	416
Expected 1 : 1	383.5	383.5

The third backcross showed clear segregation into (a) *aridum* anthocyanin in petal, spot, and filaments, and (b) the recessive colourless class. The numbers still show a deficiency in the coloured class. Fertility, however, was completely established.

*The fourth backcross.* Backcrosses were made between several third backcross heterozygotes and *hirsutum* recessive. Summarized, the results were:

Series	<b>S<sup>ARI</sup></b>	<b>s<sup>ARI</sup></b>
1	701	1116*
2	261	177
3	188	215
4	258	243

\* Not including 46 definite pure *hirsutum* natural selfs.

Here it will be seen that the ratio of **S<sup>ARI</sup>** to **s<sup>ARI</sup>** is not invariably 1 : 1, since there is a considerable excess of recessives in series 1 and 3. An excess of **S<sup>ARI</sup>** plants is present in series 2, but in series 4 there is a good 1 : 1 ratio. It need not be doubted that the anthocyanin complex of *aridum* behaves as a single gene and that it can be transferred to a *hirsutum* background.

(a) *Genetic relationship between **S<sup>ARI</sup>** and **R<sup>H</sup>** (*hirsutum* red) (fourth backcross).*

Some of the third backcross plants were heterozygous for **R<sup>H</sup>**, **R<sup>B</sup>** or **R<sup>A</sup>**, and these were backcrossed to **r<sup>H</sup>** (*hirsutum* recessive) in order to see whether linkage existed between any of these genes and **S<sup>ARI</sup>**. The results are given in Tables 2, 3 and 4.

The results of Table 2 indicate clearly that the anthocyanin complex of *aridum* behaves as a single genetic difference which can be transferred to *hirsutum* by repeated backcrossing, although with considerable

diminution in intensity. When so transferred, **S<sup>ARI</sup>**, as well as *Armourianum* spot **S<sup>ARM</sup>**, is found to be a member of a multiple allelomorphic series for anthocyanin pigmentation. This series now comprises four members, **R<sup>H</sup>**, **S<sup>ARM</sup>**, **S<sup>ARI</sup>**, and **r<sup>H</sup>** (**s<sup>H</sup>**). It will be noted that a few double recessives are present. This is explained by the fact that the backcrosses were done in the field and not in the greenhouse. It has seldom been

Table 2. *Linkage relationship between R<sup>H</sup> and S<sup>ARI</sup> in backcrosses to recessive hirsutum (fourth backcross)*

Parent	Family	<b>R<sup>H</sup>S<sup>ARI</sup></b>	<b>R<sup>H</sup>s<sup>ARI</sup></b>	<b>r<sup>H</sup>S<sup>ARI</sup></b>	<b>r<sup>H</sup>s<sup>ARI</sup></b>
38/G 19-231	126 A	0	4	0	0
	126 B	0	54	36	2
	127	0	35	14	0
	128	0	67	52	1
	129	0	19	18	0
	Total	0	179	120	3*
38/G 19-242	130	0	14	11	0
	131	0	58	24	2
	132	0	23	20	0
	133	0	61	43	1
	134	0	83	71	0
	135	0	126	62	16
	136	0	225	136	11
	137	0	13	9	1
	138	0	25	22	3
	139	0	51	30	2
	140	0	178	114	8
	141	0	80	39	2
	Total	0	937	581	46*

\* Definitely established on close examination to be *hirsutum* natural selfs.

possible to obtain crosses from the field without some self-pollination, probably due to thrips or ants. The rogues were examined and all proved to be typical *hirsutums* of the parental type. In this instance the presence of other segregating characters enabled the rogues to be picked out with complete accuracy and it is quite certain that there is no evidence of crossing-over.

(b) *Genetic relationship between S<sup>ARI</sup> and R<sup>B</sup> (barbadense red) (fourth backcross).*

Out of the cross C 542 (**R<sup>B</sup>** transferred to Upland) × C 331 two heterozygous plants **R<sup>B</sup>S<sup>ARI</sup>** **r<sup>B</sup>s<sup>ARI</sup>** were selected to cross with **rs<sup>ARI</sup>** (ordinary *hirsutum* recessive). The results are given in Table 3.

Table 3. *Linkage relationship between R<sup>B</sup> and S<sup>ARI</sup> in backcrosses to recessive hirsutum*

Family	<b>R<sup>B</sup>S<sup>ARI</sup></b>	<b>R<sup>B</sup>s<sup>ARI</sup></b>	<b>r<sup>B</sup>S<sup>ARI</sup></b>	<b>r<sup>B</sup>s<sup>ARI</sup></b>
<i>hirsutum</i> × 339	68	28	55	49
<i>hirsutum</i> × 546	65	42	73	58
Total	133	70	128	107

Here it is seen that while the ratio of  $R^B$  to  $r^B$  is approximately 1 : 1 (133 : 128) in the  $S^{ARI}$  class, it is considerably distorted (70 : 107) in the  $s^{ARI}$  class. Similarly, there is some deviation from the 1 : 1 ratio of  $S^{ARI}$  :  $s^{ARI}$  in the  $r^B$  class (128 : 107), the excess being in favour of the  $S^{ARI}$  class. The ratio of  $S^{ARI}$  :  $s^{ARI}$  in the  $R^B$  class shows a considerable excess of the former. Since  $R^B$  and  $R^H$  have been previously established to be independently inherited, and  $S^{ARI}$  is a multiple allele at the  $R^H$  locus, it is expected that  $R^B$  and  $S^{ARI}$  will be independently inherited. If linked, the repulsion type of ratio is expected. There is, however, a decided tendency for  $R^B$  and  $S^{ARI}$  to be associated, but without cytological examination and further genetical studies it is not possible to state the reason.

(c) *Genetic relationship between  $S^{ARI}$  and  $R^A$  (Asiatic red) (fourth back-cross).*

This series of experiments is important, since for the first time a gene from the Asiatic 13-chromosome genom is brought into contact with a gene from the North American 13-chromosome genom. The results are given in Table 4.

Table 4. *Linkage relationship between  $R^A$  and  $S^{ARI}$  in backcrosses to recessive hirsutum*

Families	$R^A S^{ARI}$	$R^A s^{ARI}$	$r^A S^{ARI}$	$r^A s^{ARI}$
162-172	11	17	11	7
	10	9	7	8
	30	43	35	38
	20	27	24	25
	2	8	5	3
	0	3	4	1
	4	2	1	8
	9	1	2	2
	4	1	2	3
	0	3	7	6
Total	90	114	98	101
Expected	107.5	107.5	107.5	107.5

Here it is evident that  $R^A$  and  $S^{ARI}$  are independently inherited, as was expected, although the number of  $S^{ARI}$  plants is slightly below expectation.

(3) *Mutation in the aridum anthocyanin complex  $S^{ARI}$*

In what has been said previously the symbol  $S^{ARI}$  refers to the *aridum* anthocyanin complex, viz. purple petal, coloured filaments, and strong petal spot. Up to and including the second backcross there was no reason to regard  $S^{ARI}$  as other than a unit character, and, as we have seen, behaving as an allele at the  $R^H$  (*hirsutum* red) locus. In the linkage

experiments with  $R^H$  none of the 1116 recorded  $R^H$  plants had any trace of the  $SARI$  complex. Nevertheless, in some of the third backcross cultures a number of plants were observed to exhibit a loss of a part of the  $SARI$  complex. There were two types of loss, which will be separately discussed.

#### A. Loss of petal spot leaving flush.

The most frequent type was manifested as a disappearance of both petal spot and filament colour leaving the flower with the characteristic *aridum* anthocyanin flush and with unpigmented or very faintly pigmented filaments.

Plants were recorded in which the disappearance of petal spot took place in (a) all the flowers, (b) one or two branches only, or (c) a section of a flower. In the last type, several cases were noted in which two or three petals were intensely spotted and the rest devoid of spot. Opposite to the spotted petals, the column and filaments were strongly pigmented, while opposite to the unspotted petals the filaments and column were colourless or nearly colourless.

*Genetical behaviour of somatic mutants of third backcross, crossed to recessive hirsutum.* (1) Plants with flush, but no petal spot and little or no colour in the filaments. The results are given below:

Third backcross parent	$SARI$ Flush with		$sARI$
	Petal spot and coloured filaments	No petal spot and colourless or faintly coloured filaments	No flush, spotless petal, uncoloured filaments
1712	0	15	20
1727*	40	10	42
1975	2	7	9
1978	0	30	14

\* This plant had distinctly coloured filaments.

(2) Plants with flush, spotted petals and coloured filaments, but with one or more branches possessing flowers devoid of spot and with little or no colour in the filaments (somatic mutations).

Third backcross parent	$SARI$ Flush with		$sARI$
	Petal spot and coloured filaments	No petal spot and colourless or faintly coloured filaments	No flush, spotless petal, uncoloured filaments
1704	1	14	21
1707*	0	30	27
1717	42	18	75
1719	1	9	6

\* This plant had a flower in which three petals were strongly spotted (grade 16) and two petals unspotted.



(3) Plants with flush, spotted petals, and coloured filaments, in which somatic mutation was not observed to occur.

	<b>SARI</b> Flus with		<b>sARI</b>
Third backcross parent 1812	Petal spot and coloured filaments 35	No petal spot and colourless or faintly coloured filaments 4	No flush, spotless petal, uncoloured filaments 29

In regard to the genetical behaviour of the three classes, the most important points are:

In section 1, plants with flush but no petal spot, four families were grown. Two families, 1712 and 1978, produced plants without petal spot, but only flush. Mutation in the parent plants had thus extended to the germ cells as well. In the other two families, 1727 and 1975, the somatic mutation from spot to spotless in the parents evidently did not extend wholly to the germ cells, since a variable number of plants produced petal spot.

In section 2, the results are similar to those of section 1. In three families, 1704, 1707, and 1719, mutation from spot to spotless in the parents had evidently been practically complete, two plants only possessing petal spot. In the third family, 1717, the mutation from spot to spotless had embraced only a part of the germ cells of the parent.

In section 3, plants not showing somatic mutation, the one family studied, 1812, showed 4 plants out of 39 which had mutated from spot to spotless. We have in the above families 258 **SARI** to 243 **sARI**; a close approximation to the 1:1 ratio. All families, whether the parent was spotted or unspotted, show a variable percentage of mutants from spot to spotless, and it is thus clear that part of the *aridum* colour complex is intensely mutable on a *hirsutum* background.

#### B. Loss of flush leaving petal spot and coloured filaments.

Another type of loss in the *aridum* colour complex was recorded, but far less frequently. In this the characteristic flush practically disappeared from the corolla, leaving a strong petal spot and coloured filaments. This type of loss was observed in seven plants of selfed third backcross families from plants of composition **RA<sup>SARI</sup> rASARI**. The complete results of progenies of eight plants are given in Table 5.

From Table 5 it will be seen that the group **SARI** could be divided into two groups: (1) flush+spot+coloured filaments (46 plants), and

(2) no flush + spot + coloured filaments (7 plants) (the second type of loss).

Table 5. Results of self-fertilizing third backcross plants of constitution  $R^{ASARI} r^{ASARI}$

Parent	$R^{ASARI}$ and $R^{ASARI}$	$r^{ASARI}$		$r^{ASARI}$
		(1)	(2)	
C 3178	9	5	0	0
C 3206	91	20	3	9
C 3223	73	12	2	14
C 3155	7	3	1	1
C 3168	14	4	1	2
C 3198	7	1	0	0
C 3206	3	1	0	0
C 3215	6	0	0	0
Total	210	46	7	26
		53		
Expected 12 : 3 : 1		54		18

\* (1) flush + spot + coloured filaments; (2) no flush + spot + coloured filaments.

A single plant exhibiting the second type of loss was crossed with recessive and gave results as follows:

	No flush, spot, coloured filaments	No flush, no spot, colourless filaments
Expected 1 : 1	11 12.5	14 12.5

It will be seen from the combined results that the *aridum* colour complex, which behaved as an indivisible unit as regards its linkage relationship with  $R^H$ , since no  $R^H$  plants possessed any trace of the  $S^{ARI}$  complex, breaks up into two subtypes, type 1 (flush, no petal spot), and type 2 (no flush, petal spot). Type 1 clearly originated by mutation and the  $S^{ARI}$  complex is thus analogous to  $S^P$  (*purpurascens* petal spot), which has been shown to be mutable on a *hirsutum* background (Harland, 1937a). The mode of origin of type 2 is less clear, since it has not been seen as a transitional somatic mutation. When isolated it behaves as a simple genic difference.

The breakdown of  $S^{ARI}$  into two components is capable of two explanations. Either there are two genes so close together that they behave as a single gene allelomorphous to  $R^H$ , or else  $S^{ARI}$  is a single gene which is unstable on a *hirsutum* background and can mutate to two other alleles in the colour series.

Some uncompleted experiments in which the *aridum* colour complex is being transferred to *barbadense* by repeated backcrossing indicate that on a *barbadense* background, mutation to type 1, involving loss of

filament colour and petal spot, either does not occur at all, or occurs only rarely, whereas mutation to type 2, loss of flush, leaving spot and filament colour, occurred as a single plant in one culture. These experiments, so far as they go, strengthen the view that we are dealing with one locus for the colour complex and not two, and that the frequency and direction of mutation at this locus are determined by the genetical background to which it is transferred.

#### DISCUSSION

There is little to add to what has already been said in the body of this paper, but it is convenient to emphasize the main points of importance.

First, the evidence for the hypothesis that the New World cultivated cottons are amphidiploids with one 13-chromosome genom of Asiatic affinities and the other 13-chromosome genom of North American affinities is enormously strengthened by the demonstration that alleles of  $c^R$  and  $R^H$  ( $S^{ARI}$  and  $S^{ARM}$ ) exist in the North American diploids, and an allele ( $R^A$ ) of the  $R^B$ - $S^B$  multiple allele series similarly exists in the Asiatic diploids. The alleles of crinkled established to exist in the North American diploids differ between themselves in dominance potency to the same extent as do the *hirsutum* and *barbadense* alleles (Harland & Atteck, 1940). The *Thurberi* allele resembles the *hirsutum* allele in being incompletely dominant on a *barbadense* background, whereas the *Armourianum* allele is apparently completely dominant on this background, thereby resembling the *barbadense* normal allele. The fact that a normal allele conferring complete dominance to crinkled exists in one of the diploid components, is of extreme cogency in any discussion regarding the mechanism by which dominance to a mutant is attained. The ancestral lines of *Armourianum* and *barbadense* or *Thurberi* and *barbadense* can scarcely have intermingled, since the period at which the New World tetraploids were synthesized from their primitive diploid components was probably (Harland, 1935a) at the close of the Upper Cretaceous period. According to Hutchinson & Ghose (1937) the origin of dominance in *barbadense* is ascribed to recent modification of heterozygotes as a result of the commercial cultivation of this species. It is, however, now more than likely that the history of the attainment of dominance to crinkled in the New World species stretches back in geological time for several million years, and it seems highly probable that the normal allele of great dominance potency of *barbadense* was a constituent of the original North American diploid genom.

This line of speculation leads further to the strong suspicion that the six New World amphidiploids have descended from more than one primitive amphidiploid. It may be assumed provisionally that the group comprising *hirsutum*, *purpurascens*, *punctatum*, and *Taitense* contain a component on the *Thurberi* line of ancestry, while *barbadense* and *Darwinii* may trace back to an *Armourianum-Harknessii*-like component.

#### SUMMARY

1. Genes from three North American diploid species of *Gossypium* with  $n=13$  chromosomes (*Thurberi*, *Armourianum*, and *aridum*) were transferred by repeated backcrossing to the amphidiploid  $n=26$ -chromosome species *barbadense* and *hirsutum*.

2. All three species possess normal alleles of the *barbadense* crinkled mutant  $C^R$ . *Thurberi* has a normal of weak dominance potency corresponding to that found in *hirsutum*, while *Armourianum* has a normal of strong dominance potency corresponding to that found in *barbadense*. The dominance potency of *aridum* normal appeared to be less than that of *Armourianum*.

3. The *Armourianum* petal spot,  $S^{ARI}$ , was not reduced in size or intensity on a *hirsutum* background, and on a *barbadense* background was increased in both size and intensity. It proved to be at the same locus as the anthocyanin gene  $R^H$  (*hirsutum* red).

4. The *aridum* anthocyanin colour complex  $S^{ARI}$  was reduced in intensity on a *hirsutum* background and also proved to be at the same locus as  $S^{ARM}$  and  $R^H$ . Although behaving as an indivisible unit in linkage relations with  $R^H$ , the colour complex became mutable on a *hirsutum* background in the third and subsequent backcrosses. Loss of spot and filament colour leaving the purple flush was the commonest type of mutation, but a second type, loss of flush leaving petal spot and coloured filaments, was also recorded. This phenomenon is regarded as being most probably due to mutation in two different directions of a single gene as a result of transference to a new species background.

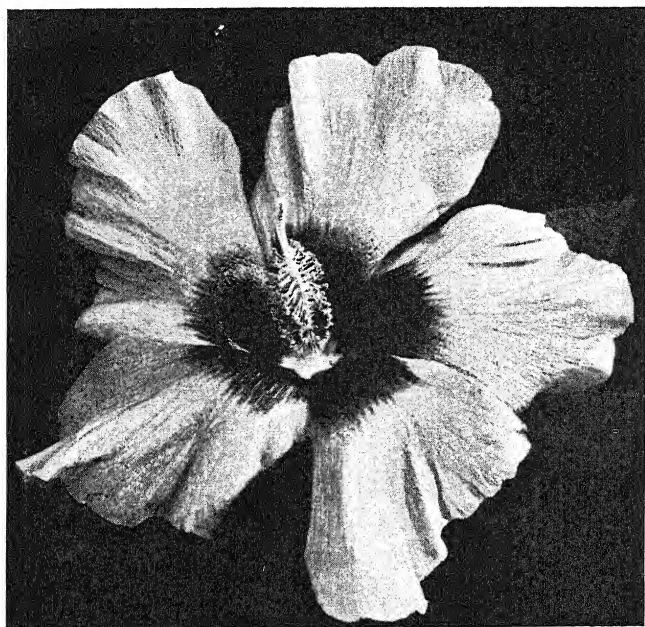
5. The demonstration that  $S^{ARI}$  and  $S^{ARM}$  of *aridum* and *Armourianum* are at the same locus as  $R^H$ , provides a conclusive proof that the amphidiploid New World cottons contain two  $n=13$ -chromosome genomes of Asiatic and North American affinities respectively.

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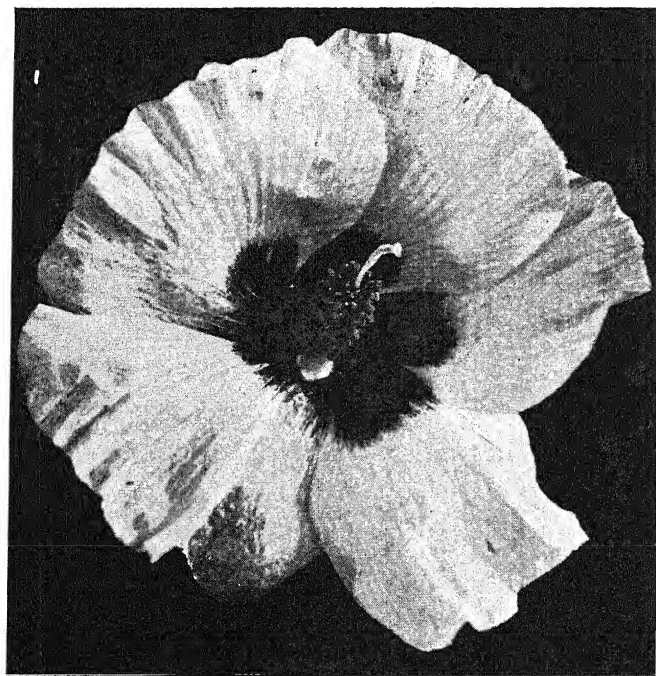
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H



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## EXPLANATION OF PLATES 1 AND 2

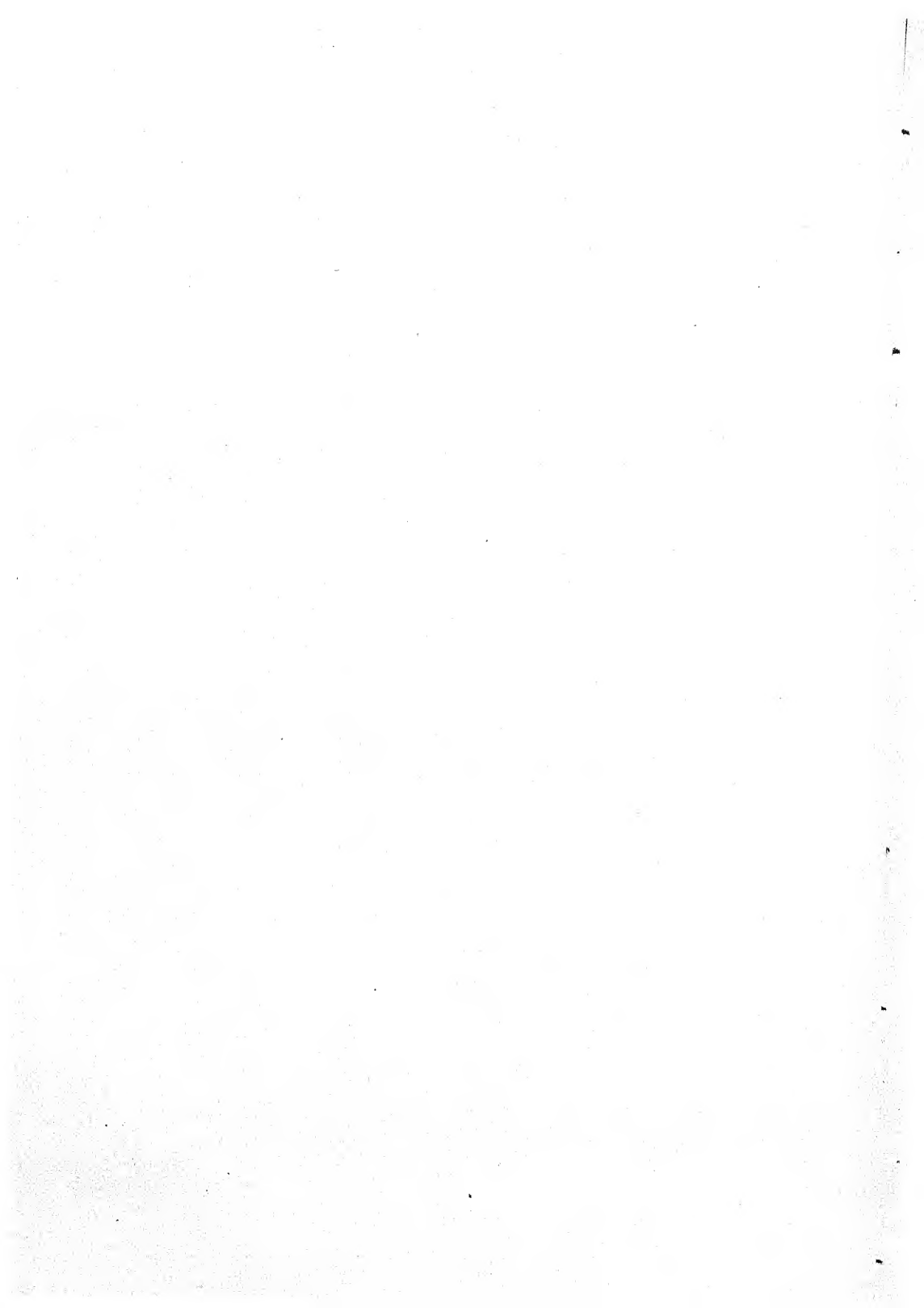
### PLATE 1

- A. Single petal of *barbadense*  $\times$  *aridum*  $F_1$ , showing the *aridum* colour complex.
- B. Single petal of second backcross plant (*barbadense*  $\times$  *aridum*)  $\times$  *barbadense*  $\times$  *barbadense*, showing diminution in size of spot and intensity of flush.
- C. *aridum* flush on a *hirsutum* background; a fourth backcross plant. This is the first type of mutation in which petal spot is lost, leaving only flush.
- D. *aridum* petal spot on a *hirsutum* background; a fourth backcross plant. This is the second type of mutation in which flush is lost, leaving only petal spot.
- E. *aridum* colour complex combined with Asiatic  $R^A$  (red flush).
- F.  $F_1$  of *barbadense*  $\times$  *Armourianum*. The spot is large and the petal small.
- G. *Armourianum* petal spot on a *hirsutum* background (fourth backcross). Note the much greater intensity of *Armourianum* spot compared with that of *aridum* (D).

### PLATE 2

- H. Flower of  $F_1$  *barbadense*  $\times$  *aridum*, showing large petal spot, coloured filaments and practically sterile anthers.
- I. Flower from a second backcross plant (*barbadense*  $\times$  *Armourianum*)  $\times$  *barbadense*  $\times$  *barbadense*, showing great development of petal spot and coloured filaments.

The paintings have been made by Miss Olga M. Atteck.



# THE GENETICS OF COTTON

## XIX. NORMAL ALLELES OF THE CRINKLED MUTANT OF *GOSSYPIUM BARBADENSE* L. DIFFERING IN DOMINANCE POTENCY, AND AN EXPERIMENTAL VERIFICATION OF FISHER'S THEORY OF DOMINANCE

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## INTRODUCTION

THE Crinkled Dwarf mutant of Sea Island cotton (*Gossypium barbadense* L.) has been the subject of much genetical investigation by the writer (1916, 1918, 1932, 1933, 1935, 1936) and by Hutchinson & Ghose (1937). Certain conclusions regarding the existence of normal alleles at the crinkled locus differing in dominance potency were presented in preliminary form (1936), thus: "It has been shown that *barbadense* possesses one allele of crinkled which may be termed  $C^{RB}$ , and that *hirsutum* has two more different alleles, namely,  $C^{RM}$  (in *hirsutum* var. Meade T9) and  $C^{RH}$  (in *hirsutum* var. Triumph T57). These three alleles are distinguishable only by their dominance reactions on various crinkled genetical backgrounds, and from incompleting experiments it is already clear that further different normal alleles are present in the other four species." In the same paper the writer's views on dominance in relation to the crinkled mutant were summarized in a single sentence: "The dominance relation is due to the interaction of a normal allele of specific potency with a modifier complex to which it is precisely adjusted, and that the genes constituting the dominance modifier complex have been preserved not because of their function as modifiers of initially disadvantageous heterozygotes but because of selective value on their own account." These views have now been modified.

As early as 1932 it was established that the crinkled heterozygote was indistinguishable from the normal homozygote not only in *barbadense*, where it occurs naturally, but also in three different types of *hirsutum*. Since that date it has been further shown that transference to a wild type of *purpurascens* (var. *Morrilli* Cook & Hubbard), to which it was transferred by repeated backcrosses, also results in complete dominance of normal. It was further established that the modifiers of dominance were *not identical* in the three species, since crosses between any two of the three species crinkleds gave in  $F_2$  a series of imperceptibly graded crinkleds ranging from semi-lethal (supercrinkled) to phenotypically normal (pseudo-normal).

The further question then arose: are the *normal* alleles of crinkled alike in different species of New World *Gossypiums*? Using various types of homozygous or nearly homozygous crinkled as bases, it became possible to transfer various normals on to them by repeated backcrossing. The crinkled bases upon which work was begun comprised *barbadense*, four different types of *hirsutum*, *purpurascens* var. *Morrilli*, and four different homozygous grades of crinkled extracted from the  $F_2$  of *barbadense* crinkled by *hirsutum* crinkled. The experiments here reported are only a

small part of those started in 1932, but, nevertheless, place on record the detailed evidence for the existence of normal alleles of differing dominance potency in New World cottons and serve as a basis for discussion of the dominance mechanism in so far as it concerns the crinkled mutant. Hutchinson & Ghose (1937), adhering to the Fisher theory of dominance, have dismissed my previous findings (1936) of alleles with differing dominance potency as inconclusive, although presented only in the form of a brief abstract of part of the present data, and put forward their view in these words: "It is clear that the main cause of dominance is groups of modifiers present in the rest of the genotype and is independent of differences at the crinkled locus."

The object of these experiments, then, is to present the results of transference by backcrossing of the normal allele of some species of New World cottons to various crinkled backgrounds, in order to demonstrate the existence of different degrees of dominance potency in such alleles, and in order to evaluate current theories of dominance.

## MATERIAL AND METHODS

### MATERIALS

#### (1) *Types of Upland cotton* (*G. hirsutum* L.)

Type 8. A variety of naked-seeded Upland collected by the writer in 1929 in a commercial field in North Carolina. It has been self-fertilized every year since 1928.

Type 9. An especially long-linted selection from the variety Meade, self-fertilized since 1925, i.e. for more than thirteen generations, since at times two generations were grown in a single year. Suspected to contain *barbadense* modifiers.

Type 34. A dwarf Upland with khaki lint grown by native Indians in Guatemala. It was obtained from the United States Department of Agriculture in 1930 through the courtesy of Dr T. H. Kearney.

Type 57. A type selected for high lint index from the commercial variety Triumph in 1928 and afterwards self-fertilized for not less than six generations.

Type 459. A selection from the Upland variety King, collected in South Carolina by the writer and self-fertilized for four generations.

#### (2) *Types of Bourbon cotton* (*G. purpurascens* Poir.)

Type 12. A typical *purpurascens* with red leaves, found as a single plant in a garden in Trinidad in 1923. Bred true on being introduced into pedigree culture, so far as eye judgement could determine.

Type 196. This type was described by Cook & Hubbard (1926) as *G. Morrilli*. It was collected from the west coast of Mexico. Comparison with an extensive collection of *purpurascens* types led to its inclusion in this species.

(3) *Taitense Parl.*

Type 77. The standard type of *G. Taitense* Parl., the endemic species of the Fiji Islands. Seeds were obtained from Mr R. A. Anson in 1929, and a single plant was subsequently maintained by occasional selfing.

(4) *Darwinii Watt*

Type 2127. Seeds of *G. Darwinii* were collected in the Galapagos Islands, where the species is endemic, and a few were given to the writer by the United States Department of Agriculture. The particular plant used in this series of experiments possessed two genes not known to exist in any other New World species, viz.  $R^D$  (red plant body) and  $Y^D$  (yellow corolla).

(5) *tomentosum Nutt.*

Type 22. The endemic species of the Hawaiian Islands. Seeds were collected by the writer in 1925 in the Island of Oahu, and the type maintained by grafting until 1933, when experiments were begun with this species.

(6) *Crinkleds used as backgrounds*

The following crinkleds were used:

(a) Type 4, *G. barbadense* (Sea Island). This is the original crinkled mutant which has been used in all experiments since 1932. The strain used in the present experiment was self-fertilized for ten or more generations.

(b) Type 9, crinkled. This crinkled was obtained by ten repeated backcrossings to *hirsutum* type 9 with subsequent extraction by selfing.

(c) Type 57, crinkled. Obtained by eight repeated backcrossings to *hirsutum* type 57, and then extracted by selfing.

(d) Type 250, crinkled. The crinkled corresponding to *hirsutum* type 250 (virescent yellow). Obtained by a similar process.

(e) Grade 1, super-crinkled. Obtained from the interspecific cross *barbadense* type 4 (crinkled)  $\times$  *hirsutum* type 9 (crinkled). This was the extremest and least improved crinkled which occurred in the  $F_2$  of the above cross. It was maintained by self-fertilization for eleven generations, and preserved its uniformity.

*Grading of crinkled.* The above five types of crinkled can be arranged according to the series of grades mentioned in a previous paper (1935).

Grade 1: the extreme form of crinkled (super-crinkled).

2: more improved.

3: the grade of type 9 *hirsutum* crinkled.

4: the grade of *barbadense* type 4 crinkled.

5: crinkled moderately developed.

5.5: the grade of the heterozygote of *hirsutum* type 9.

6: crinkled fairly well developed.

6.5: faint trace of crinkled.

7: normal.

*hirsutum* crinkled T57 was about 5.5-6.

*hirsutum* crinkled T250 was also about 5.5-6.

Passing from grade 7 to grade 1, the most pronounced changes are that the leaves become progressively more crinkled or rugose and develop chlorophyll-deficient areas giving a mosaic appearance; the edges of the leaves become more and more ragged, and the size of the leaf (and plant) smaller. The growth and vigour of the plant is also much reduced until grade 1 is a dwarf plant a few inches high with minute flowers and bolls with only two or three seeds instead of the normal complement of 18-40.

## METHODS

As a starting-point a single  $F_1$  plant was selected at random from an  $F_1$  culture grown from a single crossed boll (*circa* thirty plants).

For each successive backcross usually one boll was used, giving rise to twenty or more plants. If segregation into a normal (heterozygous) group and recessive crinkled group was visible when the plants were a few inches high, a preliminary separation was made about a month after sowing, and two to four random heterozygotes were retained for backcrossing. In other cases the separation could be made only at a much later period. The choice of the heterozygotes was always made to obviate any bias in favour of any particular grade of crinkled or of any special vigour, selection being strictly random. As regards vigour, the plants were grown in 6 in. pots in a special uniform compost, producing well-developed plants. In one or two isolated instances lack of material restricted the choice to one or two plants, but this was rectified in succeeding generations.

After the fifth backcross the plants were grown either outdoors or in



beds in the greenhouse, but the same precautions were taken to ensure a random sample of heterozygotes for backcrossing.

### THE EXPERIMENTAL RESULTS

#### (1) TRANSFERENCES OF NORMALS TO *BARBADENSE* CRINKLED TYPE 4

##### A. *Transferences of hirsutum normals*

##### A 1. *hirsutum* type 8.

The results of this experiment are given in Table 1.

Table 1. *Grade of heterozygote in repeated backcrosses of type 8 hirsutum normal to crinkled barbadense (type 4)*

Family	Grade of heterozygotes					Remarks
	5	5.5	6	6.5	7	
$F_1$	—	—	—	6	—	
Backcross 1	—	—	—	4	—	
2	—	—	—	3	—	
3	—	—	—	5	1	
3	—	—	—	3	—	
4	4	—	—	—	—	Old plants
4	4	—	—	—	—	Old plants
5	—	—	—	4	—	
6	—	—	—	2	—	
7	—	—	—	1	—	
8*	—	—	10	30	2	2 months old plants
8*	42	—	—	—	—	5 months old plants

\* There were 48 heterozygotes and 49 type 4 crinkled in this backcross, a close approximation to a 1 : 1 ratio. Six plants of the heterozygous group were stunted through bad soil conditions and were not examined.

From the results of Table 1, and generally speaking from the results of all the backcrossing experiments, it appears to be unnecessary to carry the backcrossing beyond the fourth or at most the fifth backcross, as the heterozygote is by then stabilized on the new genetical background. To meet any possible criticism, however, this experiment was continued to the eighth backcross. The heterozygotes of this backcross were examined at two stages in the life history in order to trace variation in the grade of crinkled with age. Examination of the plants at 2 months old, i.e. when just beginning to flower, gave forty plants perceptibly crinkled and two plants phenotypically normal. A later examination at 5 months old showed that all the plants were extremely crinkled, about grade 5 (just above the grade of *barbadense* mutant). It thus appears that while there is a definite failure of dominance of the *hirsutum* normal allele in young plants, the failure becomes extremely pronounced with age.

To sum up: The normal allele of *hirsutum* type 8 cannot produce complete dominance on a *barbadense* background, and therefore cannot be identical with the *barbadense* allele  $C^{RB}$ , which does produce complete dominance. It may be considered as a weaker allele at the same locus, and following the method of indicating a species allele by a superscript (1939) may be termed  $C^{RH}$ .

#### A 2. *hirsutum* type 34.

The results of experiments in which normal of type 34 was transferred to the *barbadense* crinkled background are given in Table 2.

Table 2. *Grade of heterozygote in repeated backcrosses of type 34 hirsutum normal to crinkled barbadense (type 4)*

Family	Grade of heterozygotes					Remarks
	5	5.5	6	6.5	7	
$F_1$	—	—	—	—	—	6 plants recorded as inter-crinkled
Backcross 1	—	—	—	—	—	2 plants recorded as inter-crinkled
2	1	2	1	—	—	On old plants
3	—	—	4	—	—	On old plants
4	1	—	—	6	—	1 plant grade 5—old plant in field
5	—	—	—	2	—	
5	—	—	—	2	—	
6	—	—	16*	18	—	
6	All of grades 5-5.5†					

\* Examined at 2 months old.

† Examined at 5 months old.

Essentially they are similar to those of the previous section. It will be noted that the grade of crinkled in mature heterozygotes (5 months old) is about 5-5.5, thus closely resembling *barbadense* crinkled, being, however, superior to the latter in height and vigour. The normal allele of type 34, therefore, is similar in dominance potency to that of type 8. A careful comparison of the heterozygotes of type 8, with those of type 34, gave the impression that the latter were definitely more crinkled. It cannot yet be concluded that the normal alleles of types 8 and 34 are different, since the expression of crinkled in heterozygotes is sensitive to other influences besides age, and the technique for distinguishing slight differences in dominance potency have not yet been fully worked out.

#### A 3. *hirsutum* type 57.

The results of experiments with type 57 are given in Table 3.

Table 3. *Grade of heterozygote in repeated backcrosses of type 57 hirsutum normal to crinkled barbadense (type 4)*

Family	Grade of heterozygotes			
	5.5	6	6.5	7
$F_1$	—	—	—	2
Backcross 1	—	1	—	2
1	—	1	2	—
2	1	1	1	—
3	—	1	2	2
3	2 plants recorded only as "hets"			
4*	—	3	15	2
4*	—	15	17	—

\* Ex third backcross selfed (two families).

The results of experiments with type 57 were carried only as far as the fourth backcross. The fourth backcross was not made directly from third backcross material, as the crosses failed to germinate. Fortunately, three selfed families were available from selfing of third backcross hets, and two plants were selected to cross with *barbadense* crinkled for the fourth backcross. One plant was recorded as crinkled, but behaved as a typical het when crossed with *barbadense* crinkled, while the other was recorded as normal (grade 7) and was evidently a homozygote, since it produced a family of thirty-two plants, all distinctly crinkled of grades 6 and 6.5, when crossed with *barbadense* crinkled. In general, the results from this series of crosses fall into line with those of the two previous experiments, and it may be concluded that type 57 also possesses the weak normal allele  $C^{RH}$ .

#### A 4. *hirsutum* type 459.

The results of experiments with type 459 are given in Table 4.

Table 4. *Grade of heterozygote in repeated backcrosses of type 459 hirsutum to crinkled barbadense (type 4)*

Family	Grade of heterozygotes		
	6	6.5	7
$F_1$	—	3	—
Backcross 1	—	4	—
2	2	1	—
3	1	3	—
4	—	4	1
5	—	—	1
5	—	2	—
5	2	—	—
6	7*	35	1
6	11*	32	1

\* Plants examined at 2 months old. When 5 months old the grade of crinkled of the hets diminished to 5-5.5, similar to the grade of the three *hirsutum* hets just discussed.

From the results of Table 4 it is evident that beginning with only a slight failure of dominance in  $F_1$ , the failure is repeated in successive backcrosses until in the sixth backcross eighty-five plants out of eighty-seven exhibited the typical slightly crinkled heterozygote at 8 weeks old. As in previous experiments the grade at 5 months old approximated to the grade of the recessive *barbadense* crinkled, or a little above it (grade 5-5.5).

To sum up: This series of experiments, in which the normal allele of crinkled in four different types of *hirsutum* was transferred by up to eight backcrosses to the background of *barbadense* crinkled, has given a complete demonstration of the failure of dominance in the heterozygote. Crinkling is clearly visible in young plants, but is so strongly marked in old plants as to approximate to the grade of the *barbadense* crinkled itself. The normal allele of *hirsutum*, thus established to be of weaker dominance potency than that of *barbadense*, has been termed  $C^{RH}$ , though it is believed that differences in dominance potency exist among the four types of *hirsutum* worked with. In the writer's previous communication it was stated that the normal alleles of *hirsutum* types 57 and 9 were different. Further evidence regarding the relative potency of the alleles of types 57 and 9, which substantiates this belief, will be found later in this paper.

#### B. Transferences of *purpurascens* normals to *barbadense* crinkled type 4

In this section will be considered the results of experiments in which the normal allele of crinkled in *G. purpurascens* Poir. was transferred to the background of *barbadense* crinkled.

##### B 1. *purpurascens* type 12.

The results of uncompleted experiments are presented in Table 5.

Table 5. Grade of heterozygote in repeated backcrosses of type 12 (*purpurascens*) to crinkled *barbadense*

Family	Grade of heterozygotes						
	3	4	5	5.5	6	6.5	7
$F_1$				Crinkled just visible			
Backcross 1	—	—	—	—	—	3	1
2	—	—	—	—	1	1	—
3	—	3	—	—	—	—	—
4	—	15	—	(10 tall and 5 short)			

From these results it appears that as far as the second backcross this type of *purpurascens* behaves similarly to the *hirsutum* types, i.e. showing

a slight though well-marked failure of dominance in the heterozygote. The third backcross plants, however, showed uniform and strong crinkling of the same grade as *barbadense* crinkled. The three plants of grade 4 in the third backcross were recorded as heterozygotes when young, and were selected to continue the backcrossing. Further, the parent of the third backcross was a typical heterozygote of grade 6.5. One probable but unconfirmed explanation of the results is that the normal allele of type 12 is so weakened in potency when associated with the *barbadense* modifier complex, that it is phenotypically crinkled. It was observed that the fourth backcross plants were all of the same grade of crinkled (grade 4), but that there were ten tall crinkled and five short. It seems likely that the ten tall were heterozygotes, but without repeating the whole experiment it is not possible to arrive at the correct explanation. The normal allele of type 12 is, however, demonstrated to be of weaker dominance potency than that of *barbadense*, and is comparable in this respect with the normal allele of *hirsutum*.

## B 2. *purpurascens* type 196 (var. Morrilli Cook & Hubbard).

The results of experiments involving type 196 are given in Table 6.

Table 6. *Grade of heterozygotes in repeated backcrosses of type 196 (G. purpurascens Poir. = G. Morrilli Cook & Hubbard) to barbadense crinkled type 4*

Family	Grade of heterozygotes				Remarks
	5.5	6	6.5	7	
$F_1$	—	—	4	—	
Backcross 1	—	—	3	—	
2	—	—	4	—	
2	—	—	2	2	
3	—	1	—	—	
3	3	2	—	—	Old plants
4	—	—	—	—	Not recorded
4	—	—	—	—	Not recorded
5*	—	—	4	—	
6†‡	—	15	27	4	

\* One plant began as a 6.5 at the flowering stage and the growing point subsequently became extremely crinkled.

† Ex fifth backcross selfed which gave three crinkled grade 7 and one crinkled grade 6.5.

‡ The sixth backcross plants were first recorded at the flowering stage. When examined at 5 months old, all showed extreme crinkling at the top of the plant, about grade 5.5.

The results of Table 6 indicate that in *purpurascens* (Morrilli) type 196 the normal allele is similar in potency to those found in *hirsutum*, being of weaker potency than the normal  $C^{RB}$  of *barbadense*.

Some results from selfing fifth backcross plants for two generations

are also available. First, two selfed intermediates of the fifth backcross gave:

Normal	Intermediate	Crinkled
3	1	0
4	6	4

In the next generation, progenies of all the normals and intermediates were grown. Five normals threw only normal; one (C4278) produced 11 intermediates: 11 crinkled, and another (C4256) 35 normals: 3 intermediates.

Now C4278 was classified as normal until it was 6 months old, when the growing point apparently mutated to a strong grade of crinkled (about 5). It is therefore highly probable that the complete absence of normals in its progeny can be accounted for by assuming mutation from normal to crinkled in the parental germ cells. The three intermediates of family C4256 must have resulted from the union of mutated germ cells with normal, since this plant when crossed with crinkled behaved as a normal, giving fifty-two typical intermediates.

Mutability of genes as a consequence of transference to the genetical background of another species has previously been described (Harland, 1937a), and the possibility of its occurrence in interspecific crosses has always to be borne in mind.

### C. Transference of Taitense normals to barbadense crinkled type 4

#### C 1. *G. Taitense* Parl. type 77.

The results are set forth in Table 7.

Table 7. *Grade of heterozygote in repeated backcrosses of type 77 (Taitense) to crinkled barbadense type 4*

Family	Grade of heterozygotes				Remarks
	5-5	6	6-5	7	
$F_1$		6 slightly crinkled			
Backcross 1		4 slightly crinkled			
2	—	—	2	1	Old plants
3	—	4	—	—	
4	1	2	4	—	
5*	—	8	9	—	
5*†	—	19	26	—	

\* Ex fourth backcross selfed.

† Much more crinkled in older plants (about 5-5-6).

From Table 7 it may be concluded that the normal allele in *G. Taitense* type 77 is a weaker allele as regards dominance potency than the normal allele of *barbadense*, resembling the *hirsutum* normal in this respect.

D. Transference of *Darwinii* normal to *barbadense* crinkled type 4D 1. *G. Darwinii* Watt type 2127.

The results of experiments with *G. Darwinii* are given in Tables 8 and 9.

Table 8. *Grade of heterozygote in repeated backcrosses of type 2127 (G. Darwinii) to crinkled barbadense type 4*

Family	Grade of heterozygotes		
	6	6.5	7
$F_1$	—	4	—
Backcross 1	—	—	4
2	—	2	7
3	—	—	3
4	—	1	3
5	—	—	2
5*	—	—	47
6	—	—	25
6	—	—	35

\* Ex fourth backcross selfed.

Table 9. *Grade of heterozygote in fourth backcross selfed (ex Table 8)*

Family	Grade of normal group		Crinkled
	6.5	7	
C 281	—	3	0
C 286	1	47	11
C 1993	—	6	4
C 1990	—	15	5
Total	1	71	20
	72		
Expected 3 : 1	69		23

Table 8 contains the backcross results as far as the sixth backcross, while Table 9 contains results from selfings of the fourth backcross. From the results it will be seen that after the fourth backcross the normal allele of *Darwinii* behaves similarly to the normal allele of *barbadense*, and may thus be classified as an allele of great dominance potency. The results of Table 9 are confirmatory. The results, in fact, form a complete contrast to those obtained with the weak normals of *hirsutum*, *purpurascens*, and *Taitense*. The appearance of slight crinkling in the  $F_1$  indicates that although the normal allele of *Darwinii* may be of similar dominance potency to that of *barbadense*, the two species possess different modifier backgrounds.

(2) TRANSFERENCE OF NORMALS TO *HIRSUTUM* CRINKLED TYPE 9

It has previously been established that when the *barbadense* crinkled mutant is transferred to the background of *hirsutum* type 9 (a pure line of the commercial variety Meade), the heterozygote is intermediate, and selfing of heterozygotes gives 1 normal : 2 intermediate : 1 crinkled. In contrast with this result, transference of crinkled to other *hirsutum* backgrounds has always given complete dominance of *hirsutum* normal. This has been proved to be the case with three different *hirsutums*, namely, Texas Virescent Yellow, a large-bolled selection from Triumph known as type 57, and a variety of Upland from the Punjab (type 19) which was selected from the commercial variety 285F. In addition, crinkled transferred to the background of *purpurascens* var. *Morrilli* (Cook & Hubbard) was manifested as a complete recessive. There was no sign of crinkling in the heterozygote. Hutchinson & Ghose (1937) discovered the crinkled mutant in an Indian type of *hirsutum*. Here also normal was completely dominant. It seems therefore that *hirsutum* type 9 constitutes an exception to the general condition of complete dominance, and an explanation has been put forward for this. The variety Meade from which type 9 was selected has much longer staple than any other Upland variety, and this has been ascribed to its supposed origin from a cross with *barbadense* Sea Island, which possesses extremely long staple. If type 9 contains a mixture of *hirsutum* and *barbadense* genes, and if, as has been demonstrated, *hirsutum* and *barbadense* each have dominance mechanisms genetically constructed in different ways, type 9 should exhibit failure of dominance through disharmony of modifiers. Most combinations of modifiers, on this theory, will be less efficient than the pure *hirsutum* or *barbadense* constellations.

One feature of marked importance in type 9 crinkled is the degree of crinkledness. It is more crinkled and weaker than the *barbadense* crinkled, whereas the crinkleds corresponding to ordinary *hirsutum* are more improved than the *barbadense* crinkled.

The experiments using type 9 crinkled as a background for transference are therefore important in elucidating the part played by the normal allele and the modifier complex, respectively, in producing dominance.

A. *barbadense* type 15A

The experiments may be considered in three sections:

- (1) Results of successive backcrosses (1-8).
- (2) Results from selfing plants of the seventh backcross.
- (3) Crosses of seventh backcross heterozygotes to type 9 normal.



In Table 10 will be found the results of eight successive backcrosses. It will be seen that four plants only in the whole series of experiments are recorded with a faint degree of crinkling (backcrosses 2 and 4). After the fourth backcross complete stability was arrived at: the plants showed on trace of *barbadense* characters, and the dominance of the *barbadense* normal  $C^{RB}$  was complete. It is evident, then, that the substitution of the *barbadense* normal allele for the type 9 normal allele on the back-

Table 10. *Grade of heterozygote in repeated backcrosses of barbadense normal to hirsutum crinkled type 9. Results of transference experiment barbadense normal to crinkled type 9*

Family	Grade of heterozygotes					Remarks
	5	5.5*	6	6.5	7	
$F_1$	—	—	—	—	3	3 plants recorded as "normal"; others not examined
Backcross 1	—	—	—	—	—	Grade not recorded
2	—	—	—	2	—	Slightly crinkled when old
3	—	—	—	—	6	
4	—	—	—	1	3	
4	—	—	—	1	3	
4	—	—	—	—	4	
5	—	—	—	—	5	
5	—	—	—	—	12	
5	—	—	—	—	2	
6	—	—	—	—	10	
6	—	—	—	—	5	
6	—	—	—	—	1	
6	—	—	—	—	8	
7	—	—	—	—	8	
7	—	—	—	—	1	
8	—	—	—	—	52	
8	—	—	—	—	47	

\* Indicates position of grade of heterozygote type 9 (*hirsutum*) normal  $\times$  type 9 (*hirsutum*) crinkled.

ground of type 9 converts the heterozygote from intermediate to fully normal. The difference in dominance potency of the *barbadense* and *hirsutum* normals has thus been established from yet another angle.

The results of selfing seventh backcross heterozygotes are given in Table 11. The normals were all of grade 7, with no trace of crinkled at any stage of growth, and the proportions of normal and crinkled were close to expectation.

The hypothesis that the normal alleles of *hirsutum* and *barbadense* differ in dominance potency was finally subjected to the crucial test of crossing the heterozygotes of the seventh backcross of composition  $C^{RB} c^{RB}$  with normal *hirsutum* type 9<sup>1</sup> of composition  $C^{RM}$ . This cross should give 1  $C^{RB} C^{RM}$  : 1  $C^{RM} c^{RB}$ .

<sup>1</sup> The normal allele of type 9 is designated as  $C^{RM}$  and not  $C^{RE}$ , since it is regarded as a different weaker allele.

The results of the experiment are given in Table 12. It will be seen that the two kinds of expected plants appear in approximately equal numbers, and the hypothesis of alleles of different dominance potency in *hirsutum* and *barbadense* is thus fully confirmed.

Table 11. *Results of selfing seventh backcross heterozygotes ex Table 10*

Family	Normal	Crinkled
39/G 702	23	4
G 703	43	14
G 704	40	9
G 705	24	5
G 706	22	9
G 707	42	13
G 708	48	23
G 709	24	11
Total	266	88
Expected 3 : 1	265.5	88.5

Table 12. *Results of crosses of seventh backcross heterozygotes  $C^{RM} c^R$  with hirsutum normals  $C^{RM} C^{RM}$*

Family	$C^{RB} c^R$	$C^{RM} C^{RM}$	$C^{RB} C^{RM}$ Normals	$C^{RM} c^R$ Inter- mediates (grade 5.5)
39/G 754	C 4044 × 9-C 5502		26	27
G 755	C 4045 × 9-C 5501		15	14
G 756	C 4045 × 9-C 5502		35	37
G 757	C 4049 × 9-C 5501		12	11
G 758	C 4049 × 9-C 5502		11	9
G 759	C 4052 × 9-C 5502		9	6
	Total		108	104
	Expected		106	106

### B. tomentosum

These experiments were taken as far as the fifth backcross, and the results are presented in Table 13. The chief point worthy of note is that

Table 13. *Grade of heterozygote in repeated backcrosses of type 22 (G. tomentosum) to type 9 hirsutum crinkled*

Family	Grade of heterozygotes			
	5.5	6	6.5	7
$F_1$	1	—	1	—
Backcross 1	—	1	—	1
2	—	—	2	2
3	—	Not recorded		—
4	—	—	—	2
4	—	—	—	3
5	—	—	—	44
5	—	—	—	50

the  $F_1$  showed distinct crinkling, which disappeared in subsequent generations, until in the fourth and fifth backcrosses all heterozygotes

are completely normal of grade 7. The normal allele of *tomentosum* thus resembles the *barbadense* allele in being of strong dominance potency, and differs from the *hirsutum* normal.

#### C. *hirsutum* type 57

It will be recollected that the normal allele of type 57 *hirsutum* is completely dominant to crinkled on its own background. The interest in this experiment is to see whether this dominance will be maintained on the type 9 background. The results are set forward in Table 14.

Table 14. *Grade of heterozygote in repeated backcrosses of type 57 (hirsutum) to type 9 hirsutum crinkled*

Family	Grade of heterozygotes			
	5.5	6	6.5	7
$F_1$	—	—	2	—
Backcross 1	—	—	1	2
2	—	—	1	5
3*	—	14	22	—
3*	1	28	16	—

\* Ex second backcross selfed.

Here it will be seen first that the results were carried only as far as the third backcross, and so are not absolutely conclusive, but the following points are significant. The  $F_1$  shows slight impairment of dominance, the first and second backcrosses a rather less impairment, and the third backcross a further modification in the direction of crinkled, there being one plant of grade 5.5, the grade of the type 9 heterozygote. Now whether further backcrossing would result in the heterozygote being stabilized at the same point as the type 9 heterozygote, or whether, as the writer believes from other uncompleted experiments, the normal allele of type 57 differs in potency from those of *barbadense* and type 9, cannot yet be stated with certainty. What is, however, clear, is that types 9 and 57 differ in their modifier complexes, so that while type 57 is completely dominant on its own background, it is not dominant on the type 9 background. Type 57 crinkled is much nearer normal than type 9 crinkled, and the behaviour of type 57 normal on the two backgrounds is related to the phenotypic appearance and vigour of the latter. On the bad crinkled background both type 57 and type 9 exhibit intermediacy in the heterozygote, but when the crinkled recessive is improved to the condition of type 57 crinkled, the intermediate is *correspondingly* improved, and becomes phenotypically normal.

D. *G. Darwinii*

These experiments were carried out only as far as the first backcross, as the material was lost when the work was transferred from Trinidad to Brazil. The results may be summarized for what they are worth:

$F_1$ . Two plants both grade 7 (full dominance).

First backcross. Two plants both grade 7.

So far the results agree with the hypothesis that the normal allele of *G. Darwinii* is equal in potency to that of *G. barbadense*, and greater than that of the normal alleles of *hirsutum*, *purpurascens*, or *Taitense*.

 (3) TRANSFERENCE OF NORMALS TO *HIRSUTUM* CRINKLED TYPE 57

 A. *Darwinii*

These experiments were taken only as far as the third backcross. The results are given in Table 15.

Table 15. *Grade of heterozygote in repeated backcrosses of G. Darwinii type 2127 to type 57 hirsutum crinkled*

Family	Grade of heterozygotes		
	6	6.5	7
$F_1$	—	—	4
Backcross 1	—	2	2
2	2	—	8
3	—	1†	8
3*	—	1†	11

\* Ex second backcross selfed.

† Doubtful 6.5.

From the results of Table 15 it will be seen that the  $F_1$  begins with complete dominance. There is impairment in two plants of the second backcross and complete dominance in the third backcross with two doubtful exceptions. It seems that by the third backcross equilibrium has been reached, and that the normal allele of *Darwinii* exhibits complete dominance on the background of type 57 crinkled.

 B. *hirsutum* type 9

Results as far as the third backcross are given in Table 16. From these results it is seen that type 9 normal *hirsutum* does not exhibit

Table 16. *Grade of heterozygote in repeated backcrosses of G. hirsutum type 9 to type 57 hirsutum crinkled*

Family	Grade of heterozygotes		
	6	6.5	7
$F_1$	—	1	3
Backcross 1	—	2	1
2	—	—	5
3	7	5	—
3	8	16	—

complete dominance on the background of type 57 crinkled in the third backcross, while type 57 itself does so. It is therefore almost certain that type 9 has a slightly weaker normal allele.

(4) TRANSFERENCE OF NORMALS TO CRINKLED *HIRSUTUM* TYPE 250

A. *barbadense* type 18

The results are presented in Table 17. Unfortunately, it was possible to carry the results only as far as the second backcross, but in view of the fact that the first and second backcrosses showed complete dominance

Table 17. *Grade of heterozygote in repeated backcrosses of G. barbadense type 18 to hirsutum crinkled type 250*

Family	Grade of heterozygotes		
	6	6.5	7
$F_1$	—	1	1
Backcross 1	—	—	3
2	—	—	4

it may be assumed that the *barbadense* normal allele is completely dominant on a background of type 250 crinkled. The results are therefore according to expectation.

B. *hirsutum* type 9

This experiment was expected to give similar results to those in which type 9 normal was transferred to the background of type 57 crinkled, since type 57 crinkled and type 250 crinkled show very little morphological difference.

Table 18. *Grade of heterozygote in repeated backcrosses of G. hirsutum type 9 to hirsutum crinkled type 250*

Family	Grade of heterozygotes		
	6	6.5	7
$F_1$	—	—	2
Backcross 1	—	—	4
2	—	1	3
3	—	—	12
4	—	1	27

The results are given in Table 18. As will be seen, the results as far as the fourth backcross show that complete dominance ensued when the type 9 normal was substituted for the type 250 normal, although as will be recollected type 9 is incompletely dominant on its own background. This is in marked contrast to the results previously obtained

where the substitution of type 9 for type 57 on a *hirsutum* crinkled type 57 failed to give complete dominance. It is clear that the type 9 allele must be of similar potency to the type 250 normal. The attainment of dominance is here a question of modifiers, improvement of crinkled being reflected in a corresponding improvement of heterozygous normal.

(5) TRANSFERENCE OF NORMALS TO SUPER-CRINKLED GRADE 1

Since the background of super-crinkled represents an association of an unfavourable group of modifiers combined with  $c^R$ , and is the least viable and least improved of all crinkled forms, it was thought to be of great interest to transfer various normals to this background. It was believed that such transference would constitute a powerful method of revealing differences in dominance potency of normals. The results are only fragmentary, but present some points of interest. They are given in Table 19.

Table 19. *Results of transference of normal alleles to super-crinkled*

Type of norma	Cross	Grade of crinkled of heterozygotes					
		3	4	5	6	6.5	7
<i>hirsutum</i> type 9	$F_1$	—	6	2	—	2	—
<i>hirsutum</i> type 57	$F_1$	—	—	—	2	2	2
<i>barbadense</i> type 18	$F_1$	—	—	—	—	—	3
<i>hirsutum</i> type 9	Backcross 1	3	4	—	—	—	—
<i>hirsutum</i> type 57	Backcross 1	—	1	1	—	—	—
<i>barbadense</i> type 18	Backcross 1	—	—	—	1	2	3

The main conclusions to be drawn from the table are as follows: The grade of  $F_1$  is as expected from previous experiments if three normal alleles of differing dominance potency are concerned. It is barely possible that the three parental types possess modifier complexes which may override differences in potency of the normal allele. That this is probably not so is shown by the results of the first backcross. These plants contain roughly 75% of super-crinkled modifiers, and it is clear that not only are the relative differences in dominance potency of the normals strongly brought out, but that in presence of an excessively weak modifier complex even the powerful *barbadense* allele is not able to produce complete dominance in three plants, although it was able to do so on all the backgrounds previously studied. A further significant point is that the grade of crinkled of the heterozygotes may be lower than that of the original *barbadense* mutant recessive, which was grade 4. Future work on the dominance potency of normal alleles in different species should be performed using super-crinkled as a background. To sum up: These experiments, although incomplete, indicate once more that *hirsutum* and

*barbadense* differ in the dominance potency of their normal alleles, and the conclusion previously arrived at respecting the greater potency of the *hirsutum* type 57 allele, as compared with that of *hirsutum* type 9, is strengthened.

In Table 20 the data of the preceding tables is summarized. The differences in dominance potency exhibited by the various normal alleles

Table 20. Summarized results of grade of heterozygote in all the transference experiments

Type of crinkled background	Type of normal	Grade of heterozygotes						
		3	4	5	5.5	6	6.5	7
<i>barbadense</i> type 4	<i>hirsutum</i> type 8			x — x				
	<i>hirsutum</i> type 34			x — x				
	<i>hirsutum</i> type 57					x — x		
	<i>hirsutum</i> type 459			x — x				
	<i>purpurascens</i> type 12		x					
	<i>purpurascens</i> type 196			x — x				
	<i>Taitense</i> type 77				x — x			
	<i>Darwinii</i> type 2127							x
<i>hirsutum</i> type 9	<i>barbadense</i>							x*
	<i>barbadense</i>							x
	<i>tomentosum</i>							x
	<i>hirsutum</i> type 57			x — x		x — x		
	<i>Darwinii</i>							x
<i>hirsutum</i> type 57	<i>hirsutum</i> type 9					x — x		
	<i>barbadense</i>							x
	<i>hirsutum</i> type 57							x†
<i>hirsutum</i> type 250	<i>barbadense</i>							x
	<i>hirsutum</i> type 9					x — x		x
	<i>hirsutum</i> type 250							x†
Super-crinkled‡	<i>barbadense</i>					x — x		x
	<i>hirsutum</i> type 57			x — x				
	<i>hirsutum</i> type 9		x — x					

\* Previous experiment recorded elsewhere (1916).

† Previous experiment recorded elsewhere (1933).

‡ Results of first backcross only.

on different crinkled backgrounds are clearly shown. The normals may be grouped as follows:

The normal of strongest dominance potency is *barbadense*, since it exhibits full dominance on all backgrounds except that of super-crinkled, where dominance is slightly impaired in the first backcross. The normals of *tomentosum* and *Darwinii* can be placed with *barbadense*, since both are completely dominant on the weak type 9 crinkled background.

*Hirsutum*, *purpurascens*, and *Taitense* form a distinct group with normals of weak dominance potency. This grouping is in accordance with what is known of their taxonomic relationships. Within this group it is almost certain that differences in dominance potency exist, although as previously stated it is not easy to measure them. The normal of

type 57 is more potent than that of types 9, 34, or 8, while *purpurascens* type 12 seems to be of weaker potency than any of these. When the results are considered as a whole, it is likely that the number of normal alleles at the crinkled locus may be very large. The evidence for discriminating between the alleles of types 9 and 57 is considered sufficient to assign different genetic symbols to them,  $C^{RM}$  (type 9) and  $C^{RH}$  (type 57).

#### DISCUSSION

Two main schools of thought have put forward genetical explanations for the existence of the phenomenon known as dominance. According to Fisher (1928*a*, 1928*b*, 1930, 1931), dominance is the result of an evolutionary process by which an initially disadvantageous and intermediate mutant is elevated towards normal by the accumulation of a series of modifying factors until it becomes indistinguishable from it both physiologically and phenotypically. This theory has been criticized by Wright (1929, 1934), Haldane (1930, 1939), Harland (1933, 1936), Muller (1932), Silow (1939) and others. The main opposition may be expressed as the "factor of safety" hypothesis, according to which alleles "having an activity well above the necessary minimum will be of advantage to the organism" (Dobzhansky, 1937). Haldane (1930) made the important suggestion that plus mutations at any locus may be favoured by selection provided that the original gene is not completely dominant, since the original type of gene is at a disadvantage and the new gene is not. Fisher (1931) accepted this as supplementing the mechanism of dominance evolution which he originally suggested, though he still believed that accumulation of modifiers by the heterozygote played the most important part.

The experiments described in this paper have been conducted almost continuously since 1929, with the aim of throwing light upon the methods by which dominance over a mutant is attained. A survey of the evidence presented in this paper will show that this object has been largely achieved, that the views of the two schools can be reconciled, and that there are in fact two methods by which dominance can be attained. One of these is the Fisher method; the other is the Haldane method.

An important reason for the writer's previous non-acceptance of the Fisher theory was that complete dominance was the rule in *hirsutum*, where the mutant had not been recorded, and where it was not believed to occur. This objection has been removed by the discovery of the crinkled mutant in *hirsutum* by Hutchinson & Ghose (1937). They found



it not only to be fully recessive, but also to be considerably ameliorated. This amelioration they ascribe to the conditions prevailing during the domestication of this species, though, as will be explained later, there are strong reasons for believing this view to be incorrect. The second main argument against the Fisher theory was the fact that there were two processes involved. First, the accumulation of modifiers by the heterozygotes, and secondly, the spreading of these modifiers throughout the species so that it became homozygous for them. It appeared to be impossible to imagine any mechanism which could accomplish this in self-fertilized plants (Haldane, 1939), other than a strong selective advantage of the heterozygous phase over either of the homozygous phases. With the demonstration here presented, that in three species out of six examined, dominance has been attained by the use of a strong normal allele (the Haldane effect), this objection is less cogent. It will, however, have to be assumed that the main, if not the only method of attaining dominance in a self-fertilized species, or one predominantly so, is the Haldane method. Haldane (1939) has discussed the dominance mechanism in inbred and outcrossed species. Considering species which are predominantly self-fertilized, he points out that the majority of non-lethal mutants are present in homozygotes and few in heterozygotes. Consequently, the intensity of selection for dominance must be less in inbred than in outbred species. He shows that dominance is just as common in inbred as in outbred species. The data which he presents, however, must have little bearing on whether the Fisher effect actually occurs, since he does not take into account the dominance mechanism first proposed by him, and here demonstrated to be of much more than casual importance.

#### THE HALDANE EFFECT

The evidence for the attainment of dominance by this method appears to be unshakeable. In the first place it has been shown that failure of dominance occurs in the genotype of type 9 *hirsutum*, the heterozygote being a strict intermediate. When its normal allele  $C^{RM}$  is displaced by the *barbadense* allele  $C^{RB}$ , the intermediate is converted to a full dominant at one step. Further evidence is given in Table 12, where the intermediate heterozygotes containing  $C^{RM}$  are clearly distinguishable from those containing  $C^{RB}$ , about half of each type being present according to expectation. A final piece of evidence is the fact that  $C^{RB}$  can retain much of its dominance potency even on the background of the extreme supercrinkled form.

Considering the six species worked with, it is concluded that *barbadense*, *tomentosum*, and *Darwinii* contain normal alleles of the Haldane type, and this is consistent with what is known of their taxonomic relationships.

#### THE FISHER EFFECT

This constitutes the main type of dominance construction. Here a normal allele of weak dominance potency interacts with an unknown but probably large number of modifiers to produce dominance. This type of weak normal allele has been demonstrated to exist not only in *hirsutum* but also in *purpurascens* and *Taitense*. This grouping is again in accordance with what is known of the taxonomic affinities of these species. It was first shown that the fully dominant  $Aa^1$  phase of *barbadense* was converted into an intermediate  $Aa$  phase by simply substituting the normal alleles of *hirsutum*, *purpurascens*, or *Taitense*. Next, the modifiers of *hirsutum* type 250, which conferred complete dominance in this type, were made to substitute those of type 9, which, as will be recollected, produced an intermediate heterozygote. The result was not only conversion of the intermediate heterozygote to complete dominance, but a *simultaneous and considerable improvement of the recessive*. Now in the case of *barbadense*, dominance being attained at one step, the relatively unimproved recessive must signify that direct selection pressure to improve it has not been of any great consequence, and has had little effect. This argument is the key to the situation, for if it be assumed that selection pressure on the recessive has not been dissimilar in *hirsutum* and *barbadense*, the dissimilarity in the stage of improvement of the *hirsutum* recessive can be due only to selection pressure acting on either the  $Aa$  or  $AA$  phases. Now since there is no reason to suppose that the  $AA$  phase is other than neutral towards modifiers of crinkled, the *progress of the intermediate  $Aa$  phase towards normality must have been the outcome of direct selection pressure upon the heterozygote*. This process is that required by the Fisher theory, and experimental verification of this theory may now be considered complete.

Hutchinson & Ghose (1937) have argued that the attainment of dominance in both *barbadense* and *hirsutum* has been a recent consequence of domestication. They also noted that the modification of crinkled in the direction of normal had gone much further in *hirsutum* than in *barbadense*, and considered this to be due to the fact that *hirsutum* has

<sup>1</sup>  $Aa$  = heterozygote,  $AA$  = homozygous normal,  $aa$  = homozygous recessive.

been longer in cultivation as an annual than *barbadense*. Apart from the explanation just given—that the accumulation of modifiers by the weak **Aa** phase in *hirsutum* necessarily improves the recessive—it can be shown on other grounds that the conditions of domestication can have had nothing to do with the attainment of dominance. First, crinkled transferred to the background of *G. purpurascens* var. *Morrilli*, a truly wild type from the sand dunes of the west coast of Mexico, gave not only a completely dominant heterozygote but also a recessive similar to that of *hirsutum* in its grade of improvement. It has also been established that *Morrilli* has a weak normal allele of similar dominance potency to that of the weak *hirsutum*. A second point: it has been found (1940) that two wild Mexican 13-chromosome diploids, *G. Armourianum* and *G. Thurberi*, contain normal alleles at the crinkled locus, which can be transferred to *barbadense* crinkled by repeated backcrossing. When transferred, *Armourianum* proved to have a normal allele of great potency, similar to *barbadense*, and *Thurberi* one of weak potency, similar to that of *hirsutum*. The history of dominance at the crinkled locus may thus extend back for many millions of years to the original diploid component of the New World group of tetraploids.

It has been observed that there may be a slight disturbance of dominance in some inter-*barbadense* crosses. This is true not only in Egyptian normal  $\times$  Sea Island crinkled, but also in *G. barbadense* var. *brasiliense* (Trinidad Red Kidney)  $\times$  Sea Island crinkled; and in crosses between a Peruvian type of *barbadense* (Tanguis) and Sea Island crinkled. Hutchinson & Ghose (1937) believe this slight failure to signify that the last stages of the evolution of dominance of normal in *barbadense* may have taken place in the last century, since the separation of Egyptian from the Perennial  $\times$  Sea Island hybrid population from which it was selected. They deduce further that if crinkled is transferred to the background of a *barbadense* tree cotton which has not been under cultivation, dominance should break down. Since these preliminary observations, further work has established that in the case of Egyptian normal  $\times$  Sea Island crinkled, or Sea Island normal  $\times$  Egyptian crinkled, the slight disturbance of dominance is found only in the  $F_1$ . Complete dominance is restored even in the first backcross. There is thus no reason to anticipate failure of dominance in primitive tree cottons, especially when it has been shown that the potent *barbadense* normal is nearly dominant even on the super-crinkled background.

The slight impairment of dominance in some inter-*barbadense* crosses is capable of a relatively simple explanation. Assuming dominance to be

very ancient in *barbadense* on account of the existence in the allied *Armourianum* diploid species of the powerful normal allele, the dominance mechanism of *barbadense* would tend, according to the writer's theory of gene drift (Harland & Atteck, 1933), to become constructed in slightly different ways in different ecological areas. On crossing, the now divergent dominance mechanisms would tend to disintegrate each other. Egyptian and Sea Island, and possibly other *barbadense* types, possess slightly different modifier complexes, produced in response to isolation and divergent ecological conditions. Thus even when a dominance mechanism has been perfected, it is not in a static but in a dynamic condition, and the genes contributing to its make-up must be continually changing according to selection pressure tending to select alleles of the modifiers for other more important functions. The dominance relation is analogous to a useful structure. It must be of physiological value, for otherwise its construction in so many different ways can hardly be explained. But once attained it must preserve its integrity, and it cannot do this unless it possesses enough genetic variance to reconstruct its modifier system when mutation in any gene constituting such a system results in an allele strengthening some other more important physiological process, resulting in the ultimate dissemination of this new gene through the population.

There is, finally, one aspect of the Fisher effect which it is necessary to discuss, and that is the conditions under which it is likely to become operative. The first condition is that a considerable amount of outbreeding must take place to provide for diffusion through the whole species of modifiers affecting only the **Aa** and **aa** phases. This condition is provided by such genera as *Drosophila* and *Zea*, by all self-sterile plants and generally by cotton, especially in cultivated forms. Since it has been shown that crinkled modifiers simultaneously affect both the **Aa** and **aa** phases in *hirsutum*, the diffusion process would be accelerated by natural crossing between **aa** and the other two phases. It is not improbable that this has taken place in cotton. The very precise degree of adjustment of the normal allele to its modifier complex, whatever the method of dominance construction, is difficult to explain except on the assumption that the last stage of improvement of **Aa** to completely normal was effected by cross-transference of modifiers from recessives improved by direct natural selection. It is easy to understand the improvement of **Aa** to nearly normal by accumulation of modifiers, but when the stage is reached of an excessively faint degree of crinkling, it is difficult to imagine that this faint manifestation could be removed by

direct selection of heterozygotes. It seems necessary in this particular case to invoke the recessive as responsible for the last stage.

There is also evidence of an important subsidiary mechanism for diffusion of dominance modifiers, that is, the possible selective advantage of the **Aa** phase over the **AA** phase. In a previous paper (1935) the writer attempted to show that the **Aa** phase in type 9 *hirsutum* was not only not at a selective disadvantage compared with the **AA** phase, but even slightly superior. Later and more extensive experiments, of which only the summarized results are now available, demonstrated that it was only under favourable conditions that the **Aa** phase was slightly superior. It was generally inferior under bad conditions, especially with intense competition. Now under the intensely fluctuating conditions found in nature the **Aa** phase would sometimes be under stringent selection which would tend to provide a margin of safety. At other times some **Aa** plants might have a multiplication rate exceeding that of the normal, which would assist in the diffusion of modifiers throughout the species. Further experiments are obviously required on the selective value of the **AA** and **Aa** phases for a large number of different genes.

#### SUMMARY

1. The dominance relations of the crinkled mutant of *Gossypium barbadense* L. have been studied in an extensive series of backcrossing experiments, involving six species of New World cottons.

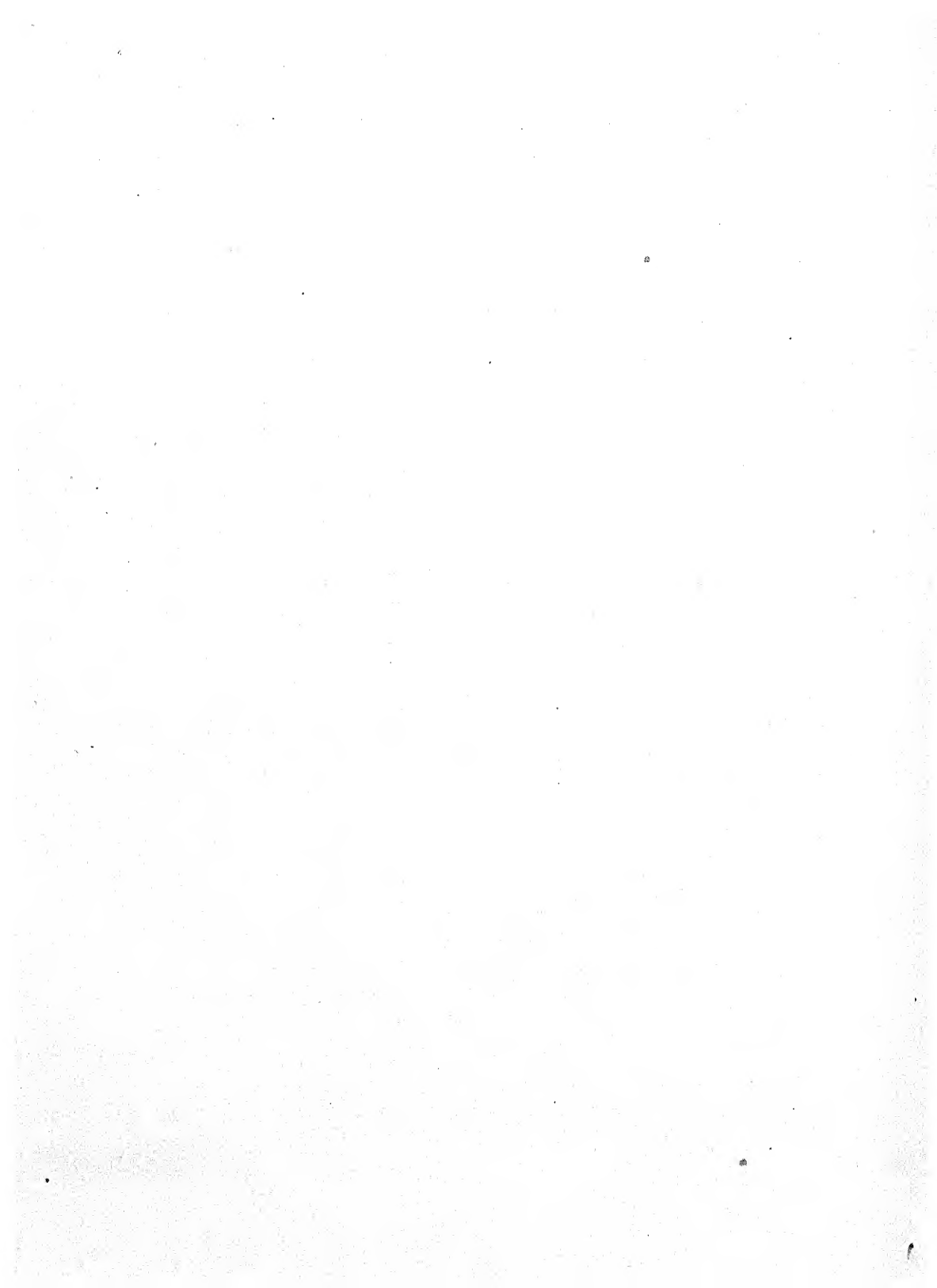
2. It is believed that there have been two methods by which dominance at the crinkled locus in the six species of New World *Gossypiums* has been attained. The first method is that proposed by Fisher, whereby dominance has been reached by modification of the heterozygous phase (*hirsutum*, *purpurascens*, and *Taitense*). The modifiers improving the heterozygous phase have simultaneously improved the recessive. The second method is that proposed by Haldane, in which dominance is attained by the employment of a normal allele of great dominance potency (*barbadense*, *tomentosum*, and *Darwinii*), the recessive phase being relatively unmodified.

3. Some conditions under which the Fisher effect is operative are discussed.

4. Evidence is brought forward indicating that the normal allele of *barbadense*, **CRB**, may become mutable on the genetic background of one type of *purpurascens*.

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# TETRASOMIC INHERITANCE IN *LOTUS* *CORNICULATUS* L.

BY C. D. R. DAWSON

(With Plate 3 and Three Text-figures)

## INTRODUCTION

HYDROGEN cyanide is known to be given off from the leaves, stems and seeds of certain plants when they are injured or killed. The chemists who investigated this phenomenon towards the end of the nineteenth century called it cyanogenesis. They found that cyanide is usually present in the form of a glycoside from which hydrogen cyanide can be liberated either by the action of an enzyme in the plant or upon the addition of dilute mineral acids.

*Lotus corniculatus* is a typical cyanogenetic plant which can give off hydrogen cyanide from its leaves and stems. When wild populations are examined, however, it is found that some individuals are incapable of liberating hydrogen cyanide. The two types of plant, cyanogenetic and acyanogenetic, are morphologically alike and grow side by side in nature. Since a high degree of self-sterility is usual in the species, both types are normally outcrossed by visiting insects.

The investigation to be described was carried out as a preliminary to a genetic analysis of wild populations. Data are presented on the inheritance of cyanogenesis in cultures of the tetraploid *L. corniculatus* and, in conclusion, the origin of the species from allied diploids is discussed.

## REVIEW OF LITERATURE

Cyanogenesis in plants has been reviewed by Robinson (1930) and by Armstrong & Armstrong (1931). Investigations have since been carried out on sudan grass (Boyd *et al.* 1938) and on a number of Australian plants (Finnemore *et al.* 1938). Crane & Lawrence (1938) have reported that, when bitter and sweet almonds are crossed, the  $F_1$  seeds are bitter.

Interest in the cyanogenetic property of *Lotus* was first aroused when transport animals were lost by poisoning during the Sudanese campaign of 1896-1900 (Henry, 1938). This led to the isolation of a substance known as lotusin from *L. arabicus* (Dunstan & Henry, 1900, 1901). A reinvestigation of this species (Henry, 1938) showed that the first



isolation was probably incorrect and that the cyanogenetic constituent of *L. arabicus* is really lotaustralin, the glucoside isolated by Finnemore *et al.* (1938) from *L. australis*. Lotaustralin has the composition  $C_6H_{11}O_5 \cdot O \cdot C(CH_3)(C_2H_5) \cdot CN$  and is thus very similar to linamarin,  $C_6H_{11}O_5 \cdot O \cdot C(CH_3)_2 \cdot CN$ , which was isolated by Jorissen & Hairs (1891) from flax.

Cyanogenesis in *L. corniculatus* was first investigated by Armstrong *et al.* (1912, 1913) in connexion with their studies on enzyme action. They found that a cyanogenetic glycoside was present in small quantity in the leaves and stems but were unable to isolate it in the pure state. They were impressed by the variability in cyanide content within *L. corniculatus* and found that individual plants had either varying amounts of glycoside in their green parts or else none at all. On the other hand, *L. uliginosus* was never found to contain even a trace of glycoside. Only one plant of *L. tenuis* was collected in Britain. When examined it was found to be rich in cyanide, as were two supposed samples of the same species from Holland and Italy. An attempt was made to correlate the variability in glycoside content of *L. corniculatus* with climatic and soil conditions (Armstrong *et al.* 1912), but as this did not give an adequate explanation it was suggested (Armstrong *et al.* 1913) "...that some other factor or factors are concerned in the production of the glucoside if not of the enzyme". A genetic control of cyanogenesis was thus hinted at as early as 1913.

Recently, Williams (1939) has shown that in *Trifolium repens* the presence or absence of cyanogenetic glycoside is determined by a single gene pair. Presence of glycoside is dominant over its absence, and modifying factors cause quantitative variation. *T. repens* is tetraploid,  $2n=32$ , compared with other *Trifolium* species,  $2n=16$  (Senn, 1938).

#### MATERIALS

When the genetic investigation was started, a collection of *Lotus* plants from different parts of Britain, Germany and Sweden was available. By the time a satisfactory crossing technique had been worked out, however, most of these had finished flowering with the exception of some of the Swedish types. Plants of Swedish origin were therefore used for crossing. They had the advantage of an erect habit, and produced a large number of flowers. They gave offspring with a high degree of self-sterility and segregated sharply into cyanogenetic and acyanogenetic classes. Some plants other than those used in the genetic investigation

gave a graded segregation when crossed. This was presumably due to the action of modifying factors.

The initial cross in the investigation was made between two plants designated as 69<sup>3</sup>/37 and 71<sup>6</sup>/37. 69<sup>3</sup>/37 was completely free from cyanide, 71<sup>6</sup>/37 was strongly cyanogenetic from the seedling stage onwards. Plant 69<sup>3</sup>/37 came from seed received from Statans Centrala Frökontrollanstalt, Stockholm (seed lot 98.094), 71<sup>6</sup>/37 came from seed received from Botaniska Museet, Lund. I am indebted to Drs H. Witte and Heribert Nilsson for sending me the seed. Both these plants had the same chromosome number as British *L. corniculatus* and gave fertile offspring when crossed with British specimens from three different localities.

The type of *L. corniculatus* used in the genetical experiments is illustrated in Pl. 3a. British plants are often more prostrate; an extreme prostrate type from the Outer Hebrides is shown in Pl. 3b for comparison.

Wild populations were sampled by means of the qualitative test for hydrogen cyanide to be described below. The general distribution of *Lotus* species in Britain is shown in Text-fig. 1. Their morphological characters are described and illustrated by Butcher & Strudwick (1930).

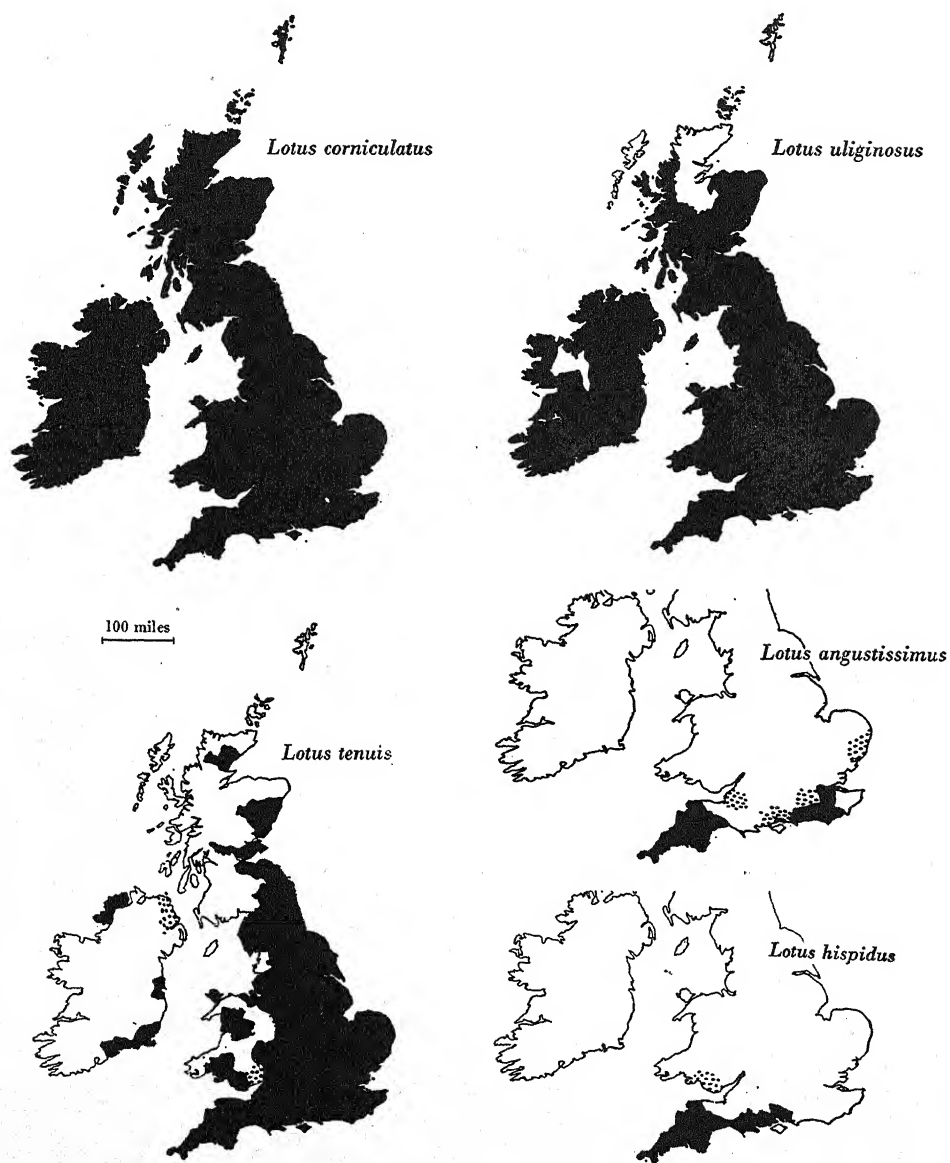
*L. corniculatus* is one of the commonest plants of pastures, commons and roadsides throughout Britain, so that material of this species was easily obtained. *L. uliginosus* is also widespread but not so common as *L. corniculatus*. It is usually found on marshy ground and in moist ditches.

Material of the three remaining species was difficult to obtain owing to their limited occurrence. Only a few populations of these have as yet been adequately tested. *L. tenuis* grows locally in Britain either near the sea or in isolated inland habitats. *L. angustissimus* and *L. hispidus* are restricted to a few southern counties and occur chiefly on cliff slopes near the sea. They are both rare but are often found growing together.

*L. corniculatus*, *uliginosus* and *tenuis* are perennials; *L. angustissimus* and *hispidus* are annuals.

#### GENETICS

(a) *Seed germination.* Like other leguminous plants *Lotus* has hard seeds. To overcome the inconvenience of delayed germination, seeds were lightly ground with fine glass paper and soaked on moist filter paper in Petri dishes. The dishes were kept at about 20° C. Any seeds which had not swollen after 6-8 hr. were picked out, dried and ground a second time. They were then put back to soak. When swollen, the seeds were



Text-fig. 1.

sown in sterilized soil. The addition of nodule bacteria to the soil made no appreciable difference to seedling growth and was discontinued.

(b) *Pollination*. Emasculation of the flowers was found to be impracticable. The damage caused by slitting the keel in the bud stage was so great that the style withered and the flower dropped off. Fortunately, it was found that the flowers were usually self-sterile. If a high degree of self-sterility can be established, it is safe to cross without previous emasculation. Strips of brass ( $13 \times 1$  cm.) were used to effect pollination. One end of each strip was covered with dark-coloured silk so that it acted as a brush on the stigma and held the pollen well. With this brush it was possible to clean a stigma of most of the pollen surrounding it and then transfer to the stigma the pollen from another plant.

(c) *Qualitative test for hydrogen cyanide*. Sodium picrate was used in testing for the presence of hydrogen cyanide. A solution of picrate was made by dissolving 50 g. of sodium carbonate in 1 l. of water. 5 g. of picric acid were added and, after stirring, the solution was filtered.

Strips of filter paper measuring  $3 \times 0.9$  cm. were saturated in the sodium picrate thus prepared, and the excess liquid removed on clean sheets of filter paper. While still moist, the strips were put in flat-bottomed tubes ( $5 \times 1$  cm.) together with three or four drops of toluene. About three leaves of the *Lotus* plant to be tested were put into the tube. It was then corked and incubated at  $25^{\circ}$  C.

The presence of hydrogen cyanide, and hence of glycoside, was shown by a reddening of the yellow picrate paper (see Pl. 3 c). The reaction was recorded after approximately 24 hr. incubation. The reddening of the picrate paper following contact with hydrogen cyanide is here called a positive reaction; failure of the picrate paper to show any change towards red after 24 hr. incubation is called a negative reaction. The terms positive and negative are also applied to cyanogenetic and acyanogenetic plants for convenience.

(d) *Experiments*. In the initial cross mentioned above,  $71^6/37$  was a strong positive and  $69^3/37$  a clear negative. Segregation occurred in the  $F_1$  generation in the following manner (Table 1):

Table 1

Parents	$F_1$	Seeds sown	Seeds germinated	Plants reaching testing stage	Reaction	
					+	-
$71^6/37 (+) \times 69^3/37 (-)$	$5/38, 5/39$	167	151	151	125	26
$69^3/37 (-) \times 71^6/37 (+)$	$4/38, 4/39$	138	135	132	83	49

The results from reciprocal crosses are significantly different,  $\chi^2$  being

equal to 14.3 for one degree of freedom, for which  $P$  is less than 0.001. When 71<sup>6</sup>/37 is the mother the ratio of positive to negative is approximately 5 : 1; when 69<sup>3</sup>/37 is the mother the ratio is nearly 2 : 1.

This reciprocal difference is most probably due to the difference in degree of self-sterility of the two parents. Table 2 summarizes the results obtained when they were self-pollinated.

Thus 71<sup>6</sup>/37 set no seed after artificial or natural self-pollination. 69<sup>3</sup>/37 gave no seed when allowed to self naturally, but when artificially selfed a total of 155 flowers set ninety-one seeds. Artificial selfing was carried out by depressing the keel of each flower so that the pollen was forced past the stigma. This is essentially the same as the cleaning operation which took place prior to making every cross. Thus a plant which was partially self-fertile had an opportunity to fertilize its own ovules every time a cross was made.

Table 2

Plant	Method of selfing	No. of heads	No. of flowers	No. of pods produced	No. of seeds produced	No. of seeds per flower	Germination %
71 <sup>6</sup> /37	Artificial	16	67	0	—	—	—
	Natural	Approx. 30	Approx. 150	0	—	—	—
69 <sup>3</sup> /37	Artificial	35	155	22	91	0.59	85.5
	Natural	9	42	0	—	—	—

Since 69<sup>3</sup>/37 is the negative parent, the most probable explanation of the reciprocal difference in  $F_1$  would be that when 69<sup>3</sup>/37 was the mother, the negative class in the offspring was increased by selfing. 71<sup>6</sup>/37 has never been observed to set seed after self-pollination, so that the results obtained when 71<sup>6</sup>/37 was the mother are likely to be more reliable. The observed segregation of 125 : 26 is a close approximation to a 5 : 1 ratio. Such a ratio would be expected if the parent plants were behaving as autotetraploids. The following crosses were made to test the hypothesis that 71<sup>6</sup>/37 was duplex (AAaa) and 69<sup>3</sup>/37 nulliplex (aaaa) for a dominant gene determining the inheritance of cyanogenetic glycoside.

(1) Eight negative plants were chosen at random from the  $F_1$  families 4/38 and 5/38 and intercrossed. These plants also served as a testing block for crossing with positives.

(2) Sixteen positive plants were chosen at random from families 4/38 and 5/38 and intercrossed. All possible combinations between them could not be made owing to the limited number of flowers produced. They were therefore arranged in four groups of three and one group of four, and all possible combinations, including reciprocals, were made between

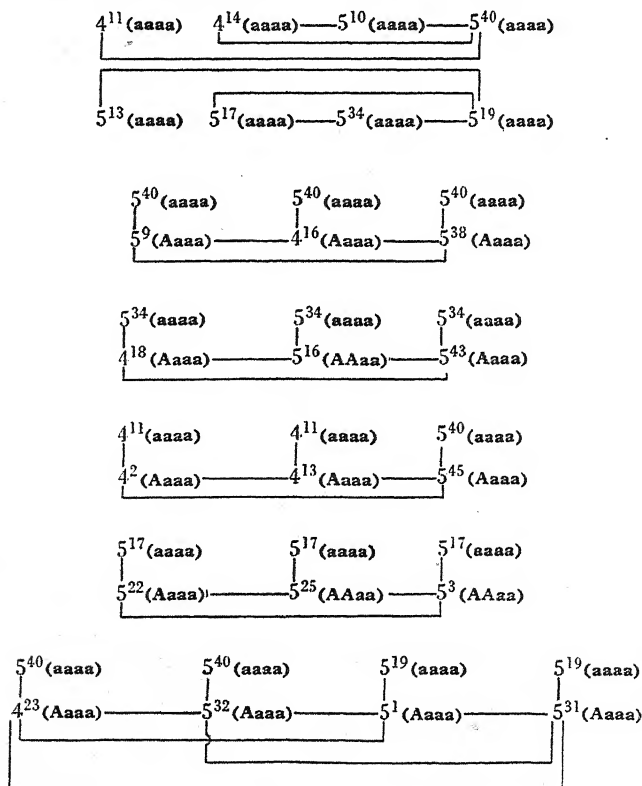
the members of each group. Each positive was also crossed reciprocally with one of the eight negatives in the testing block.

The self-sterility of all plants used for crossing was tested by artificially selfing from fourteen to forty-nine flowers on each plant. Only three of the twenty-four plants used were found to be slightly self-fertile. The results from selfing  $F_1$  plants are given in Table 3. The arrangement of

Table 3. *Result of artificially selfing the twenty-four  $F_1$  plants used for crossing*

Plant	No. of heads	No. of flowers	No. of pods	No. of seeds
5 <sup>9</sup> /38	11	49	3	9
5 <sup>45</sup> /38	6	24	1	2
4 <sup>11</sup> /38	7	33	1	0
Remaining 21 plants	139	582	0	0

the crosses is shown in Text-fig. 2 in which the assumed genotypes have been appended to the pedigree number of each plant.



Text-fig. 2.

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The  $F_2$  data are given in Table 4 and summarized in Table 5. Negative plants bred true when intercrossed and positive plants segregated in agreement with expectation when either intercrossed or crossed with negatives.

The heterogeneity for each type of cross was calculated by Brandt and Snedecor's method (Mather, 1938). Although this method was originally intended for cases where the deviation  $\chi^2$  of the totals is

Table 4

The totals for the positive and negative reactions are shown in italic type

Genotype	Cross	Seeds sown	Seeds germinated	Plants reaching testing stage	Reaction	
					+	-
<i>aaaa</i> × <i>aaaa</i> and reciprocal	<i>5<sup>40</sup></i> × <i>5<sup>19</sup></i>	40	40	27	0	27
		40	40	38	0	38
					<i>0</i>	<i>65</i>
	<i>5<sup>40</sup></i> × <i>5<sup>10</sup></i>	31	31	26	0	26
		40	39	33	0	33
					<i>0</i>	<i>59</i>
	<i>5<sup>40</sup></i> × <i>4<sup>14</sup></i>	14	14	8	0	8
		38	38	17	0	17
					<i>0</i>	<i>25</i>
	<i>5<sup>40</sup></i> × <i>4<sup>11</sup></i>	40	38	28	0	28
		40	40	22	0	22
					<i>0</i>	<i>50</i>
	<i>5<sup>19</sup></i> × <i>5<sup>34</sup></i>	40	39	34	0	34
		40	39	34	0	34
					<i>0</i>	<i>68</i>
	<i>5<sup>19</sup></i> × <i>5<sup>17</sup></i>	39	37	28	0	28
		40	40	27	0	27
					<i>0</i>	<i>55</i>
	<i>5<sup>19</sup></i> × <i>5<sup>13</sup></i>	—	—	—	—	—
		40	39	34	0	34
					<i>0</i>	<i>34</i>
	<i>4<sup>14</sup></i> × <i>5<sup>10</sup></i>	25	24	24	0	24
		40	38	38	0	38
					<i>0</i>	<i>62</i>
	<i>5<sup>34</sup></i> × <i>5<sup>17</sup></i>	40	39	39	0	39
		29	28	28	0	28
					<i>0</i>	<i>67</i>
<i>Aaaa</i> × <i>aaaa</i> and reciprocal	<i>5<sup>9</sup></i> × <i>5<sup>40</sup></i>	120	118	73	31	42
		80	77	35	21	14
					<i>52</i>	<i>56</i>
	<i>4<sup>16</sup></i> × <i>5<sup>40</sup></i>	80	78	51	27	24
		80	77	52	30	22
					<i>57</i>	<i>46</i>
	<i>5<sup>38</sup></i> × <i>5<sup>40</sup></i>	80	77	30	18	12
		80	77	45	21	24
					<i>39</i>	<i>36</i>

Table 4 (*cont.*)

Genotype	Cross	Seeds sown	Seeds germinated	Plants reaching testing stage	Reaction	
					+	-
Aaaa × aaaa and reciprocal ( <i>cont.</i> )	4 <sup>18</sup> × 5 <sup>34</sup>	120	116	96	51	45
		120	116	76	37	39
					88	84
	5 <sup>43</sup> × 5 <sup>34</sup>	51	50	41	22	19
		160	157	113	52	61
					74	80
	4 <sup>2</sup> × 4 <sup>11</sup>	80	79	74	34	40
		80	79	71	33	38
					67	78
	4 <sup>13</sup> × 4 <sup>11</sup>	80	79	66	39	27
		80	78	62	26	36
					65	63
	5 <sup>45</sup> × 5 <sup>40</sup>	80	76	69	29	40
		80	79	67	30	37
					59	77
	5 <sup>22</sup> × 5 <sup>17</sup>	80	77	71	35	36
		80	80	70	37	33
					72	69
	4 <sup>23</sup> × 5 <sup>40</sup>	40	40	27	12	15
		40	40	33	19	14
					31	29
	5 <sup>32</sup> × 5 <sup>40</sup>	40	40	28	17	11
		40	40	17	9	8
					26	19
	5 <sup>1</sup> × 5 <sup>19</sup>	40	40	23	12	11
		40	39	29	15	14
					27	25
	5 <sup>31</sup> × 5 <sup>19</sup>	40	39	35	19	16
		40	38	31	20	11
					39	27
AAaa × aaaa and reciprocal	5 <sup>16</sup> × 5 <sup>34</sup>	120	118	90	79	11
		120	118	86	73	13
					152	24
	5 <sup>25</sup> × 5 <sup>17</sup>	151	141	129	108	21
		102	98	84	71	13
					179	34
	5 <sup>3</sup> × 5 <sup>17</sup>	117	113	107	95	12
		120	117	105	86	19
					181	31
	Aaaa × Aaaa and reciprocal	5 <sup>9</sup> × 4 <sup>16</sup>	80	73	50	34
80			74	57	43	14
					77	30
4 <sup>16</sup> × 5 <sup>38</sup>		80	75	45	31	14
		80	76	47	38	9
					69	23
5 <sup>9</sup> × 5 <sup>38</sup>		80	75	41	30	11
		80	77	41	26	15
					56	26



Table 4 (cont.)

Genotype	Cross	Seeds sown	Seeds germinated	Plants reaching testing stage	Reaction	
					+	-
<b>Aaaa × Aaaa</b> and reciprocal (cont.)	$4^{18} \times 5^{43}$	173	158	143	99	44
		16	15	15	10	5
					109	49
	$4^2 \times 4^{13}$	80	78	71	57	14
		80	80	73	58	15
					115	29
	$4^{13} \times 5^{45}$	79	78	73	52	21
		82	81	70	53	17
					105	38
	$4^2 \times 5^{45}$	80	73	68	49	19
		37	29	29	22	7
					71	26
	$4^{23} \times 5^{32}$	80	79	78	62	16
		79	77	77	59	18
					121	34
	$5^{32} \times 5^1$	—	—	—	—	—
		29	27	27	19	8
					19	8
	$5^1 \times 5^{31}$	80	78	75	55	20
		40	40	40	33	7
					88	27
	$4^{23} \times 5^1$	80	78	78	64	18
		80	79	78	52	22
					116	40
	$5^{32} \times 5^{31}$	31	30	30	21	9
		58	55	55	47	8
					68	17
	$4^{23} \times 5^{31}$	33	32	32	25	7
		—	—	—	—	—
					25	7
<b>AAaa × AAaa</b> and reciprocal	$4^{18} \times 5^{16}$	120	115	104	95	9
		120	115	103	93	10
					188	19
	$5^{16} \times 5^{43}$	160	148	131	114	17
		98	89	75	68	7
					182	24
	$5^{22} \times 5^{25}$	120	119	110	102	8
		120	118	109	101	8
					203	16
	$5^{22} \times 5^3$	120	118	116	104	12
		120	118	118	111	7
					215	19
<b>AAaa × AAaa</b> and reciprocal	$5^{25} \times 5^3$	240	239	237	231	6
		165	165	159	153	6
					384	12

Table 5

Genotype	Expected ratio	No. of crosses	Observed + -	Total	Deviation			Between reciprocal totals			Between crosses			Reciprocal total/cross interaction			Between reciprocals		
					$\chi^2$	D.F.	P	$\chi^2$	D.F.	P	$\chi^2$	D.F.	P	$\chi^2$	D.F.	P	$\chi^2$	D.F.	P
aaaa × aaaa and reciprocal	0 : ∞	9	0	485	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aaaa × Aaaa and reciprocal	1 : 1	13	696	689	1385	0.03538	1	0.9-0.8	0.0624	1	0.8	8.4600	12	0.8-0.7	13.9912	12	0.5-0.3	—	—
AAaa × aaaa and reciprocal	5 : 1	3	512	89	601	1.4938	1	0.3-0.2	0.8820	1	0.5-0.3	0.3830	2	0.9-0.8	1.2132	2	0.7-0.5	—	—
Aaaa × Aaaa and reciprocal	3 : 1	13	1039	354	1393	0.1266	1	0.8-0.7	—	—	—	10.1472	12	0.7-0.5	—	—	13.0357	11	0.3-0.2
AAaa × Aaaa and reciprocal	11 : 1	4	788	78	866	0.5144	1	0.5-0.3	1.2174	1	0.3-0.2	2.9625	3	0.5-0.3	1.1782	3	0.8-0.7	—	—
AAaa × AAaa and reciprocal	35 : 1	1	384	12	396	0.09377	1	0.8-0.7	—	—	—	—	—	—	—	—	0.4995	1	0.5-0.3

Heterogeneity

significantly different from the expectation, it can also be used when the totals show no significant deviation. The factor  $n^2/(a_1 a_2)$  is not used, but instead  $n^2/(m_1 m_2)$ , where  $m_1$  and  $m_2$  are the numbers expected in the total segregation. Thus for a 1:1 segregation the factor is  $2^2/(1 \times 1)$ , for a 5:1 segregation it is  $6^2/(5 \times 1)$ .

For example, the heterogeneity of the 1:1 data is analysed as follows. There are twenty-six segregations from reciprocals, giving a total of twenty-six degrees of freedom. Thirteen of the segregations are derived from  $Aaaa \times aaaa$  matings and the other thirteen from  $aaaa \times Aaaa$ . There are two ways in which the segregations can be classified. The data from  $Aaaa \times aaaa$  matings can first be added up separately from the data from  $aaaa \times Aaaa$  matings. This gives reciprocal totals for which a heterogeneity  $\chi^2$  of one degree of freedom can be calculated. Secondly, the data can be classified into thirteen subtotals corresponding to thirteen different crosses  $[(Aaaa \times aaaa) + (aaaa \times Aaaa)]$ . For the heterogeneity between crosses there are twelve degrees of freedom. The total of the thirteen crosses gives a deviation  $\chi^2$  of one degree of freedom. Finally, the number of degrees of freedom for the reciprocal total/cross interaction is obtained by subtraction. The analysis can be set out as follows:

	D.F.
Deviation from 1:1	1
Heterogeneity: Between reciprocal totals	1
Between crosses	12
Reciprocal total/cross interaction	12
Total	26

Where the male and female parents cannot be distinguished genetically, e.g.  $Aaaa \times Aaaa$  and  $AAaa \times AAaa$ , the heterogeneity between reciprocal totals has no meaning. In such cases, the total number of degrees of freedom is divided into the deviation from expectation, the heterogeneity between crosses and the heterogeneity between reciprocals. The latter source of heterogeneity was split up in the previous example into the heterogeneity between reciprocal totals and the reciprocal total/cross interaction.

Thus in the 3:1 data (Table 4) there are twenty-four reciprocals (eleven pairs of reciprocals and two singles) and thirteen crosses. The analysis is then:

	D.F.
Deviation from 1:1	1
Heterogeneity: Between crosses	12
Between reciprocals	11
Total	24

The heterogeneity  $\chi^2$  values correspond to  $P$  values which are never as low as 0.2. The totals are therefore in good agreement with the hypothesis of tetrasomic inheritance of cyanogenesis in the *L. corniculatus* plants investigated.

In the cross (duplex  $\times$  nulliplex), an  $F_1$  segregation of

$$1AAaa : 4Aaaa : 1aaaa$$

is expected. Hence on taking  $F_1$  positives at random and crossing them with negatives it would be expected that one plant would segregate 5 : 1 to every four plants segregating 1 : 1. As seen from Text-fig. 2 and Table 4 the following result was obtained from the sixteen positive plants tested in this way:

	Giving 5 : 1	Giving 1 : 1	Total
Observed	3	13	16
Expected	3.2	12.8	16

The number of plants tested is small but the result is close to the expectation. This is further evidence in support of the tetrasomic hypothesis.

Backcrosses between a negative  $F_1$  plant ( $5^{19}/38$ ) and the parents gave the following result (Table 6):

Table 6

Cross	Seeds sown	Seeds ger- minated	Plants reaching testing stage	Reaction		Expecta- tion
				+	-	
69 <sup>8</sup> /37 (-) $\times$ 5 <sup>19</sup> /38 (-) and reciprocal	80	79	48	0	48	0 : $\infty$
71 <sup>6</sup> /37 (+) $\times$ 5 <sup>19</sup> /38 (-) and reciprocal	320	315	297	261	36	5 : 1

The result from backcrossing to the negative parent is in full agreement with expectation. The progeny from backcrossing to the positive parent showed segregation into positive and negative. There is a slight deviation from the 5 : 1 ratio expected ( $\chi^2 = 4.4$ ,  $P = 0.05-0.02$ ), but as this result was obtained from one backcross only it is insufficient to affect the probability of the conclusion reached from the  $F_2$  data.

## CYTOLOGY

### Technique

Root tips for chromosome counts were fixed in 2BE, cut at  $16\mu$  and stained in Newton's gentian violet. Buds were prefixed in Carnoy solution for half a minute and then transferred to 2BE. They were cut

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at  $16\mu$  and stained in gentian violet. Fairly good fixation of anthers was also obtained in iron acetocarmine smears, but the chromosomes could not be easily spread out by pressure on the cover-slip.

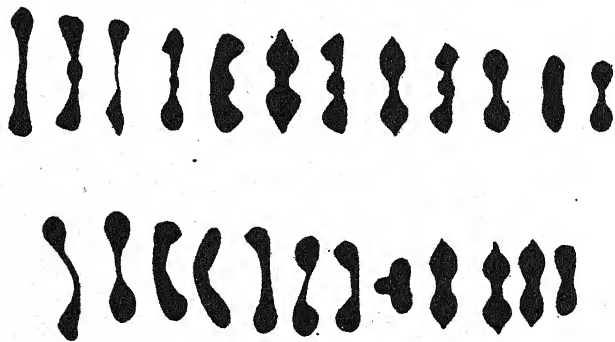
### Observations

The chromosome numbers of the five *Lotus* species native to Britain have been determined by various workers using continental material (Senn, 1938). These numbers have now been confirmed for British plants. The somatic numbers are:

	$2n$	Locality
<i>L. corniculatus</i> L.	24	Worthing, Sussex
<i>L. uliginosus</i> Schkuhr.	12	Chapel Lawn, Hereford
<i>L. tenuis</i> Wald. & Kit.	12	Midrips, Sussex
<i>L. angustissimus</i> L.	12	Braunton, North Devon
<i>L. hispidus</i> Desf.	24	Braunton, North Devon

The Swedish plants of *L. corniculatus* used in the genetical investigation also had  $2n = 24$ .

In view of the tetrasomic segregation observed, it was of interest to find out how often quadrivalents were formed in *L. corniculatus* at meiosis. Owing to the small size of the chromosomes it was not possible to obtain numerical data on this point. Until further cytological examination has been carried out, it can only be said that in the material studied, bivalents were usually formed and quadrivalents appeared to be rare. The only quadrivalent identified as such beyond doubt is illustrated in Pl. 3e. Dividing univalents were sometimes seen at first anaphase.



Text-fig. 3. Bivalents of two cells of *L. corniculatus* seen in side view at first metaphase. Carnoy 2BE.  $\times 4400$ .

Pl. 3d shows a pollen mother cell with twelve bivalents in polar view and drawings of the bivalents of two typical cells are shown in side view in Text-fig. 3.

## DISCUSSION ON AUTOTETRAPLOIDS

The importance of polyploidy has become generally recognized in recent years, but there is still much uncertainty as to the type of polyploid which is concerned in particular cases. The ease with which stable allopolyploids can often be made experimentally has sometimes led to the assumption that this type of polyploid has played the major role in the increase in chromosome number among wild plants. For this assumption to be made, however, there is as yet too little known about the genetic behaviour of wild polyploid species. On the other hand, loose definitions have led to the conclusion that autopolyploidy has been more important in evolution than allopolyploidy. Here again, although far-reaching conclusions have been made solely on the basis of cytological observation, there is not enough known about the genetics of wild polyploids. Müntzing (1936) says: "In short, the presence of multivalents indicates autopolyploidy, the absence of multivalents allopolyploidy." That this cannot be the basis for distinction between the two types of polyploid is illustrated by the well-known case of *Primula kewensis*. This experimentally produced allotetraploid has both bivalents and quadrivalents at meiosis. If such a plant were found in nature, it would be regarded on Müntzing's definition as an autotetraploid because it forms multivalents. Again, if the absence of multivalents were taken as the chief criterion of allopolyploidy, *Lotus corniculatus* might be regarded on cytological evidence as an allopolyploid. This in turn might lead to unlikely conclusions as to its origin from the diploid species with which it seems to be related on morphological grounds. However, the genetic evidence makes this conclusion improbable. Though quadrivalents are rare in the *L. corniculatus* material which was examined, the tetrasomic inheritance which was found suggests that the species should be regarded not as an *allo*- but as an *autotetraploid*. Further genetical evidence from other linkage groups is required to test this hypothesis. The attempt made by many workers to class a given species on insufficient grounds as either an *allo*- or an *autotetraploid* leaves out of consideration the possibility that some of its chromosomes may be giving tetrasomic inheritance while others may be giving disomic inheritance. The extent of each type of behaviour within a given plant can only be decided by genetic analysis. Clearly then, the most important criterion of an autotetraploid is neither the formation of quadrivalents nor the number of quadrivalents produced out of the total possible, but instead, the presence of tetrasomic inheritance in the progeny and the extent to which it occurs.

It is usually thought that autotetraploids have low fertility owing to uneven separation of chromosomes from multivalents at meiosis. This is probably due to the fact that the cytology of artificially produced autotetraploids has been studied more often than that of wild autotetraploids. The artificially produced autotetraploids have usually been kept in cultivation for short periods under the most favourable conditions and have not been exposed to the rigours of natural selection. Wild autotetraploids, on the other hand, are liable to change under the influence of selection. They have survived either (a) by developing a mechanism which gives regular disjunction at meiosis, or (b) by adopting some asexual means of reproduction.

(a) The first method whereby regular disjunction is obtained is illustrated by *Dactylis glomerata*. This species has been regarded as an autotetraploid derivative of *D. Aschersoniana* from cytological and morphological evidence (Müntzing, 1936, 1937), a conclusion which has since been supported by the genetic results of Myers (1940). Quadri-valents are formed at meiosis but they are all of the convergent and parallel types which separate evenly at first anaphase. The species is consequently highly fertile and reproduces entirely by seed. The second method whereby regular disjunction is obtained at meiosis is illustrated by *Lotus corniculatus*. Here tetrasomic inheritance denotes homology among at least four chromosomes, but nevertheless quadrivalents appear to be rare. Bivalents are formed and there is even separation at first anaphase. The species has developed no special means of asexual propagation beyond the fact that as a perennial it produces a tufted growth among the surrounding herbage. It reproduces by seed and its fertility is good.

There are no doubt numerous similar cases of autotetraploids which produce mostly bivalents at meiosis, but unfortunately, where the cytology has been investigated most thoroughly, the genetic confirmation of autopolyploidy is usually lacking. Thus in some of the sexually reproducing species of *Tulipa*, a low frequency of quadrivalents has been found ranging from 0 to 0.7 per cell out of a possible 12 (Upcott, 1939). Low frequencies of quadrivalents have also been recorded among the short chromosomes of autotetraploid animal cells where selection has never been active (*Culex*, *Schistocerca*, *Chrysochraon*). It is not surprising, therefore, to find that quadrivalents are rare in *Lotus corniculatus*. Its chromosomes are rather short, but besides this it can be assumed that they have undergone selection for the production of bivalents since the origin of the species. The number of bivalents formed in an autotetraploid

will be determined by the rate of chromosome pairing at zygotene and by the number and position of chiasmata.

(b) Sexual reproduction appears to have been frequently lost by polyploids and replaced by apomixis or some other form of vegetative propagation. This process may not have been so common among autotetraploids as among the higher polyploids because of the possibility of modifying meiosis in one of the ways mentioned above. There are, however, a few characteristic examples of autotetraploids which appear to be in the process of losing their sexual reproduction or else have lost it altogether. One of the most interesting of these is *Biscutella laevigata* (Manton, 1934). It has diploid and tetraploid forms with a different geographical distribution. The tetraploid is regarded as an autotetraploid and forms some quadrivalents at meiosis (Manton, 1934; and unpublished report in Müntzing, 1936). Meiosis cannot be quite regular, however, because plants with slightly aberrant chromosome numbers are often found in nature. In spite of this, reproduction is still largely carried out by seed, but the tetraploids have a marked tendency towards the formation of *adventitious root buds*. These have so far not been observed among the diploids. A more extreme case is met with in *Ranunculus Ficaria* (Marsden-Jones, 1935). This species has diploid and tetraploid forms which grow together in the same habitat. The diploid is sexual and reproduces entirely by seed; the tetraploid, on the other hand, is sterile but forms bulbils in the axils of its leaves which give it a ready means of vegetative propagation.

#### OCURRENCE OF CYANOGENESIS IN WILD POPULATIONS

Plants of the British species of *Lotus* were tested in several localities in order to obtain an estimate of the frequency with which cyanogenesis occurs in wild populations. The areas on which plants were tested varied somewhat in size according to circumstances, but in general the aim was to sample an interbreeding community on each particular site.

In some populations of *Lotus corniculatus* the proportion of positive and negative plants was difficult to estimate owing to the presence of plants of intermediate reaction. This was particularly so in the case of the Dorset populations on Ballard Down and Red Horn Quay, Studland. The Studland Heath roadside population probably also contained plants of intermediate reaction, but at the time it was examined the leaf samples were given a routine incubation of 24 hr. only. Plants of intermediate reaction gave a negative reaction or very slight discoloration of the picrate paper after 24 hr. A slight positive reaction then developed



during the next period of 24 hr. incubation. This might remain slightly positive or become still darker if the sample were allowed to incubate longer. It seems probable that in populations of this kind, modifying genes with quantitative effects are segregating as well as the main gene for the production of cyanogenetic glycoside. Perhaps all populations, if examined thoroughly enough, would be found to contain some plants giving intermediate reactions owing to the presence of only minute quantities of glycoside in their leaves. In many populations, however, the segregation is sharp enough to allow the proportion of the negative or homozygous recessive class to be estimated.

Plants of intermediate reaction have so far not been observed in *L. tenuis*.

Table 7 summarizes the results obtained from the examination of wild populations. The data confirm the observations made by Armstrong *et al.* (1912, 1913) on single plants of *L. corniculatus* and *uliginosus* and extend them to include the three rarer species of the genus.

*L. corniculatus* has been examined most thoroughly, but the data obtained from the other species are probably sufficient to indicate the frequencies of positive and negative types. Populations of *L. corniculatus* have always been found to contain positives and negatives. The proportion of negatives may be as low as 2 % or as high as 30–40 %. In the populations where the proportion of negatives was high, the exact number of negatives was difficult to count owing to the presence of some plants which gave a negative reaction after 24 hr. incubation and a more or less weak positive reaction on further standing.

*L. tenuis* appears to be usually positive, but one wild population and one seed lot were found to have a low proportion of negatives. *L. uliginosus* populations, on the other hand, have been found to contain negative plants only. The data on *L. angustissimus* and *hispidus* are not extensive but tend to show that in these species also cyanogenesis is rare or entirely absent.

#### DISCUSSION ON THE ORIGIN OF *LOTUS CORNICULATUS*

The British *Lotus* species can be naturally divided into the annuals and the perennials. The two annuals, *L. angustissimus* and *hispidus*, are similar in habit, geographical distribution and the absence of cyanogenetic glycoside. *L. hispidus* is tetraploid with regard to *L. angustissimus* and the two may be directly related.

The perennial species have for long been a source of trouble to

systematic botanists. *L. tenuis* and *uliginosus* have been continually confused with the much more variable *L. corniculatus*. *L. tenuis* Wald. & Kit. has been called *L. corniculatus* L. var. *tenuifolius* Rchb. and *L. uliginosus* Schkuhr. has been called *L. corniculatus* L. var. *major* Ser. and *L. corniculatus* L. subsp. *uliginosus* Pers.

In a monograph on the systematics of the genus *Lotus*, Brand (1898) ranked *L. corniculatus* and *L. uliginosus* as separate species but called *L. tenuis* a variety of *L. corniculatus*. *L. tenuis* and *corniculatus* certainly have much in common. Their flowers, calyces, fruits and seeds are almost identical and their only real distinguishing characters are the narrower leaflets and stipules and more slender stems of *L. tenuis*.

The mode of inheritance of cyanogenesis in *L. tenuis* has not yet been established for at present only the  $F_1$  generation has been raised. Negatives breed true and the  $F_1$  plants from crosses between positive and negative are all positive. It seems probable that cyanogenesis in this species will prove to be controlled by a single gene pair.

*L. uliginosus* is also perennial but has many constant differences from each of the other two species. It differs in the shape of its flowers, the greater number of flowers per head, the narrowness of the fruits, the small size and yellow colour of the seeds, the reflexed calyx teeth, the greater breadth of leaflets and stipules, and the possession of succulent runners. To these differences can now be added the presence of the dominant gene for glycoside production in *L. tenuis* and *corniculatus* and its absence in *L. uliginosus*.

As the constancy of the characters in the diploids and the variability of the tetraploid became better known, *L. tenuis*, *corniculatus* and *uliginosus* became generally recognized as distinct species. The determination of the chromosome numbers gave further support to this conclusion. Though morphologically similar, *L. corniculatus* and *tenuis* have  $2n=24$  and 12 respectively, while *L. uliginosus*, though unlike *L. tenuis* in its morphological characters, has the same chromosome number.

*L. tenuis* and *L. uliginosus* are compared with *L. corniculatus* in Table 8. L denotes that a particular character is like the corresponding character in *L. corniculatus*, U denotes that it is unlike.

Thus in seven out of ten characters *L. tenuis* is similar to *L. corniculatus*. Two others give no indication either way, since the breadth of leaflets and stipules of *corniculatus* is intermediate between that of the two diploid species, and the duration (perennial) is the same for all three species. In the remaining character the stems of *L. uliginosus* have more or less the thickness of *L. corniculatus* stems (both being rather

Table 7. *Data from wild populations*

Species	Locality	Initial incubation period of test: 24 hr.			Plants tested				Remarks
		+	-	% -		+	-	% -	
<i>L. corniculatus</i>	Ranmore Common, Surrey, I	183	145	8	5.2				Two negatives showed slight discoloration on prolonged incubation
	Ranmore Common, Surrey, II	146	138	8	5.5				Three negatives showed slight discoloration on prolonged incubation
	Crumbles, Sussex, I	155	150	5	3.2				No intermediates after prolonged incubation
	Crumbles, Sussex, II	100	98	2	2.2				No intermediates after prolonged incubation
	Pewley Down, Surrey, I	150	143	7	4.7				No intermediates after prolonged incubation
	Pewley Down, Surrey, II	150	142	8	5.3				One negative gave slight discoloration on prolonged incubation
	Portnadler Bay, Cornwall	147	134	13	8.8				No intermediates after prolonged incubation
	Studland Heath roadside, Dorset: I II III IV V VI VII VIII IX X	145	107	38	26.2				Samples from adjacent areas. Incubated 24 hr only
		97	67	30	30.9				
		203	128	75	36.9				
		76	53	23	30.3				
		124	85	39	31.4				
		133	77	56	42.1				
		52	27	25	48.1				
		37	30	7	18.9				
		118	73	45	38.1				
		100	78	22	22.0				
	Red Horn Quay, Studland, Dorset	185	119	66	35.7				After 24 hr.
	Ballard Down, Dorset, I Ballard Down, Dorset, II	185	135	50	27.0				Same sample after 72 hr.
		151	95	56	37.1				After 24 hr.
		151	114	37	24.5				Same sample after 72 hr.
		151	80	71	47.0				After 24 hr.
		151	105	46	30.5				Same sample after 72 hr.

*L. tenuis*

Mitcham, Surrey  
 Rame Head, Cornwall  
 Wallasea Island, Essex  
 Copsale, Sussex  
 Midrips, Sussex  
 Crumblies, Sussex  
 Dernford Fen, Cambridgeshire

1  
 1  
 1  
 5  
 14  
 14  
 147  
 168

1  
 1  
 1  
 5  
 14  
 147  
 166

0  
 0  
 0  
 0  
 0  
 0  
 2

1-2

—  
 —  
 Collected by Mr W. Davies  
 Collected by Mr W. Davies

Identity of negatives confirmed by growing them in cultivation  
 Seed received from Dr H. A. Senn. Not strictly a wild population

*L. uliginosus*

Seed from Mus. d'Hist. Naturelle, Paris  
 Rame Head, Cornwall  
 Downside, Surrey  
 Wisley, Surrey  
 Woking, Surrey  
 Hindhead, Surrey  
 Woking Canal, Surrey  
 Chapel Lawn, Herefordshire  
 Epping Forest, Essex  
 Cavenham, Suffolk  
 Seed from Welsh Plant Breeding Station (Ale 69)  
 Seed from Welsh Plant Breeding Station (S 121)

68  
 52  
 95  
 124  
 200  
 165  
 6  
 4  
 2  
 2  
 54

66  
 0  
 0  
 0  
 0  
 0  
 0  
 0  
 0  
 0  
 0

2  
 52  
 95  
 124  
 200  
 165  
 6  
 4  
 2  
 2  
 54

2-9

Collected by Dr D. H. Valentine  
 Not strictly a wild population  
 Not strictly a wild population  
 Not strictly a wild population

*L. angustissimus*

Seed from Vilmorin-Andrieux et Cie  
 Seed from Suttons, Reading  
 Seed from Correvoit et Fils, Geneva

59  
 59  
 15  
 28

0  
 0  
 0  
 0

59  
 59  
 15  
 28

100

Not strictly a wild population  
 Not strictly a wild population  
 Not strictly a wild population

Womersley, Surrey  
 Rame Head, Cornwall  
 Braunton, North Devon  
 Rame Head, Cornwall  
 Braunton, North Devon  
 Portmadre Bay, Cornwall

175  
 59  
 19  
 52  
 14  
 147

0  
 0  
 0  
 0  
 0  
 0

175  
 59  
 19  
 52  
 14  
 147

100  
 100  
 100  
 100  
 100  
 100

Collected by Dr Elliston Wright  
 Collected by Dr Elliston Wright  
 Tested too early in the year (27. iii. 40) to distinguish between the two species

*L. hispidus*

Mixed sample of  
*L. angustissimus*  
 and *hispidus*

variable according to genotype and habitat), while those of *L. tenuis* are usually more slender.

The broader leaflets and stipules and thicker stems of *L. corniculatus* compared with *L. tenuis* are typical of the differences often found between diploids and the tetraploids which have been artificially produced from them. Since *L. corniculatus* has most of the characters of *tenuis* and few of *uliginosus*, it would seem that *L. corniculatus* has most probably arisen as an autotetraploid from *L. tenuis* or its prototype. This view is supported by the genetic segregation observed and the fact that negative plants of

Table 8. *Comparison of L. tenuis and L. uliginosus with L. corniculatus*

	<i>tenuis</i>	<i>uliginosus</i>
Flower shape	L	U
Flower number per head	L	U
Calyx teeth in the bud	L	U
Fruits	L	U
Seeds	L	U
Succulent runners	L	U
Presence of glycoside	L	U
Duration	L	L
Breadth of leaflets and stipules	U	U
Stem thickness	U	±L

*L. tenuis* have been found in nature. *L. tenuis* itself could thus have been the source of the recessive gene for absence of glycoside in the tetraploid, and there is no reason to suppose that this has been introduced from *L. uliginosus* by allopolyploidy. The frequencies with which negative plants occur in *L. tenuis* are too high to be due to recurrent mutation alone. There is presumably a mechanism whereby the frequency of recessives is maintained, and it can be assumed that this mechanism has been passed on to the tetraploid *L. corniculatus*.

If autotetraploids can be produced artificially from *L. tenuis* (e.g. by colchicine treatment) it will be interesting to compare their morphological characters with those of *L. corniculatus*. Some divergence is to be expected between such artificial and natural tetraploids, because if *L. corniculatus* arose at some time from the prototype of *L. tenuis*, both may have undergone considerable genic change since then.

Further evidence against the origin of *L. corniculatus* by allopolyploidy is afforded by the results from species crosses. In 1939 I found plants of *L. tenuis* and *uliginosus* growing side by side on Rame Head, Cornwall. *L. corniculatus* was also growing in the same field. Bees could easily fly from one diploid species to another and effect cross-fertilization if this were possible. I attempted to cross the two diploids in cultivation

by reciprocally pollinating three *tenuis* and three *uliginosus* plants from different localities. Although 205 flowers of *tenuis* and 220 flowers of *uliginosus* were used, no hybrid seeds were obtained. The cross might succeed if carried out on a larger scale, and even if the union of haploid gametes were impossible, occasional male and female unreduced gametes might combine to give a fertile allotetraploid. However, the fact that no species hybrids have so far been obtained can be taken into consideration along with the other evidence for the autotetraploid origin of *L. corniculatus*.

#### SUMMARY

1. *Lotus corniculatus* is a north temperate plant common and widespread in Britain.
2. It exists in two forms which are morphologically indistinguishable. One form is cyanogenetic, i.e. liberates hydrogen cyanide from its leaves when they are killed, the other is acyanogenetic.
3. The hydrogen cyanide is probably combined in the plant in the form of a glycoside. A cyanogenetic glycoside has been isolated in a pure state from *L. australis* and *L. arabicus*.
4. The sodium picrate test was used for the detection of hydrogen cyanide.
5. Cyanogenesis in *L. corniculatus* was found to be determined by a dominant gene which gives tetrasomic inheritance in  $F_2$ .
6. The plants used in the genetical investigation segregated sharply into cyanogenetic and acyanogenetic classes. Some other plants were found to give off only traces of hydrogen cyanide. This is presumably due to the action of modifying factors.
7. The chromosome numbers of the perennial species in Britain are *L. tenuis* and *uliginosus*,  $2n=12$ ; *L. corniculatus*,  $2n=24$ . Those of the annual species are *L. angustissimus*  $2n=12$ ; *L. hispidus*  $2n=24$ .
8. In *L. corniculatus* quadrivalent formation is rare at meiosis.
9. It is concluded that tetrasomic inheritance must be taken as the chief criterion of an autotetraploid. The formation and number of quadrivalents are a secondary consideration.
10. *L. corniculatus* and *tenuis* have cyanogenetic and acyanogenetic forms in the same wild population. *L. uliginosus*, *angustissimus* and *hispidus* have never been found to be cyanogenetic.
11. The origin of *L. corniculatus* is discussed and its chief characters are compared with those of *tenuis* and *uliginosus*. The evidence from morphology, genetics, cytology and wild populations suggests that *L.*

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*corniculatus* has arisen as an autotetraploid from *L. tenuis* or its proto-type.

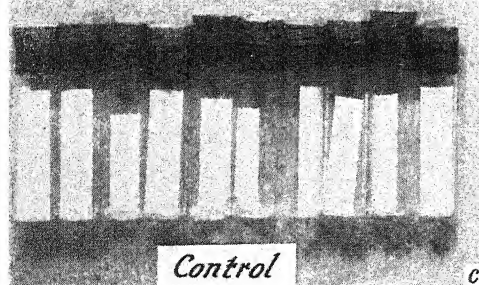
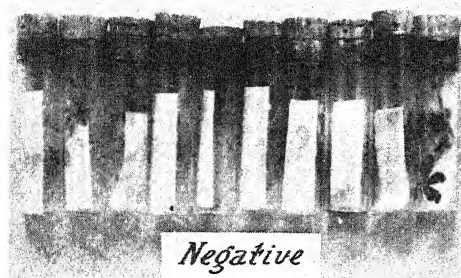
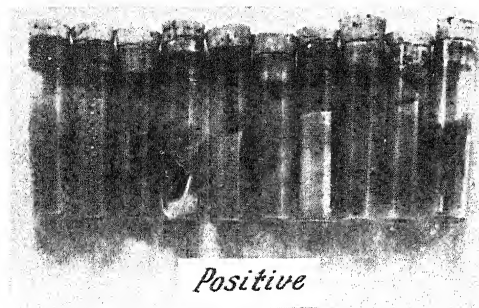
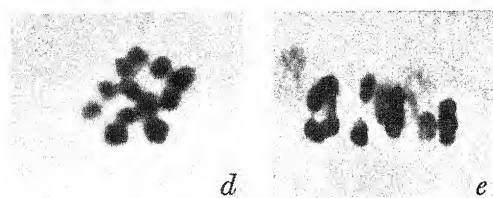
I wish to thank all those who have sent me material, Dr K. Mather for assistance with the interpretation of the results, and Captain C. Diver for his encouragement.

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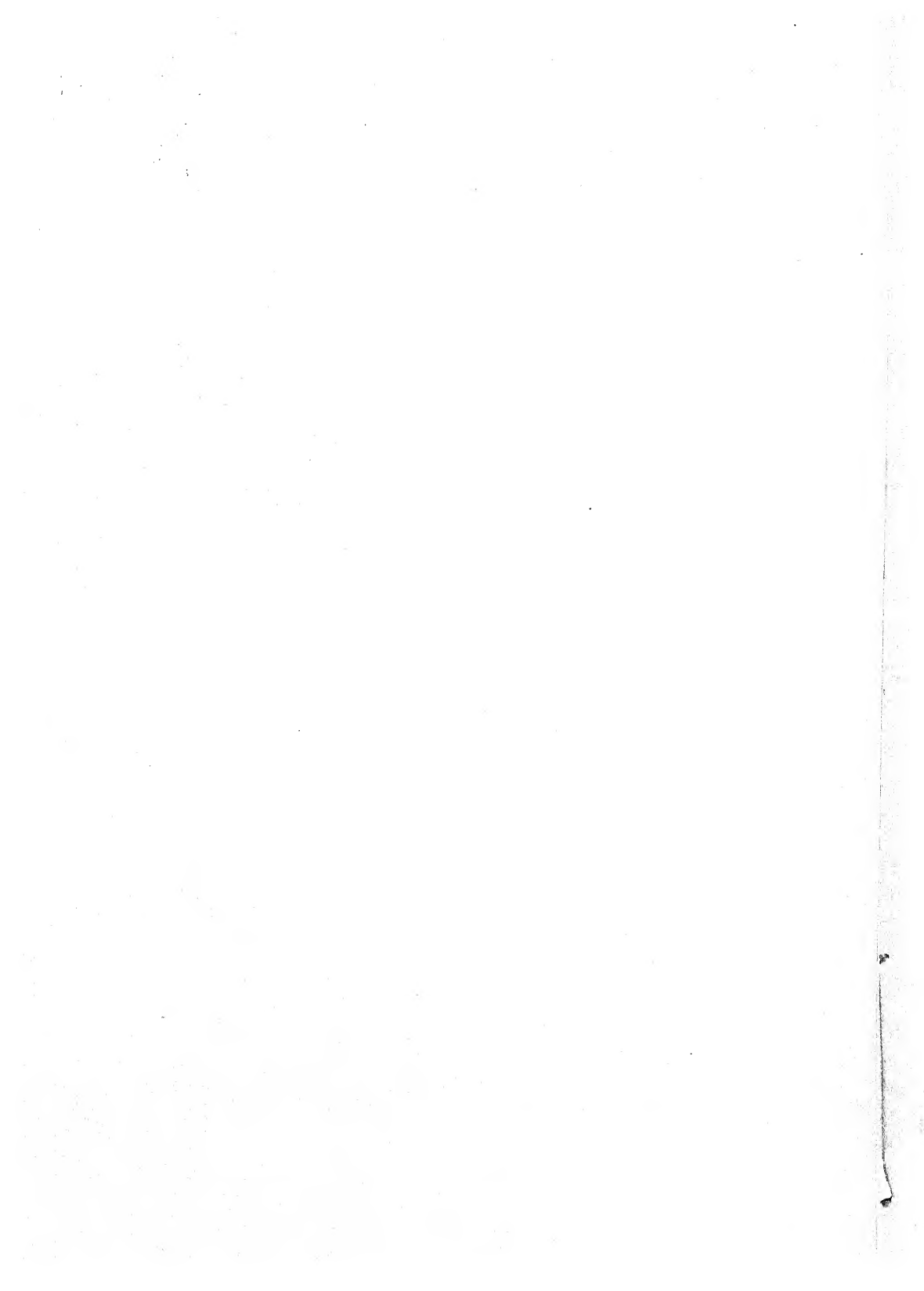
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### EXPLANATION OF PLATE 3

- Fig. a. Swedish type of *L. corniculatus* used in the genetical experiment. Reduced to the same extent as b.
- Fig. b. Prostrate type of *L. corniculatus* from Outer Hebrides. Scale in inches.
- Fig. c. The sodium picrate test for cyanogenesis in leaves of *Lotus*.
- Fig. d. Pollen mother cell of *L. corniculatus* showing twelve bivalents in polar view. Carnoy 2BE.  $\times 2830$ .
- Fig. e. Pollen mother cell of *L. corniculatus* showing quadrivalent in side view. Iron acetocarmine smear.  $\times 2000$ .







# POLLEN-TUBE GROWTH STUDIES IN CHERRIES<sup>1</sup>

By T. RAPTOPOULOS

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(With Plate 4 and Seven Text-figures)

THE aim of the present investigation is to discover the relation of incompatibility within species to that between species. In *Prunus avium*, the sweet cherry, all self-pollinations and many cross-pollinations entirely fail, owing to the inhibition of pollen-tube growth in the style. Fusion of male and female gametes is essential for fruit development and, in the numerous observations so far made, no cases of parthenocarpy or apomixis have been observed. The breeding experiments of Crane (1925-37) have revealed an elaborate system of self- and cross-incompatibility, which conforms in general with the theory of East & Mangelsdorf (1925). This system does not hold strictly for the tetraploid sour cherries, and it seemed that an investigation of the position regarding compatibility between species with different chromosome numbers might help to elucidate the working of the incompatibility mechanism in general.

The information yielded by the experiments falls under two main heads:

(1) The relative growth rates of haploid and diploid pollen in diploid, triploid and tetraploid styles.

(2) The causes of reciprocal differences.

The experiments were grouped as follows:

Part I. Pollinations made on *P. cantabrigiensis* ( $4x=32$ ).

Part II. Self-pollinations of one tetraploid and two triploid hybrids.

Part III. Pollinations made on *P. avium* ( $2x=16$ ).

## MATERIAL AND METHODS

The experiments were conducted both in the field and under glass. The diploid sweet cherry Baumann's May and the tetraploid *P. cantabrigiensis* were used as females and were self- and cross-pollinated with pollen of diploid, triploid and tetraploid varieties. The outdoor pollinations include the tetraploid hybrid 10/34 derived from *P. cantabrigiensis*  $\times$  Sour Cherry and certain triploid interspecific hybrids.

<sup>1</sup> Part I of a thesis approved for the Degree of Ph.D. in the University of London.

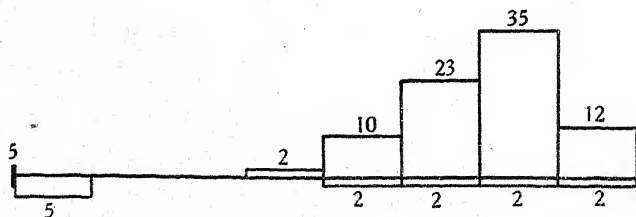
The styles were fixed in equal parts of 95 % alcohol, glacial acetic acid and lactic acid, and also in Flemming's solution, at periods from 2 to 9 days after pollination. After dehydrating and embedding in the usual way the styles were cut longitudinally at 12–18  $\mu$  and stained with haematoxylin by either Delafield's or Heidenhain's method; the first was more successful with *C. cantabrigiensis* and the second with diploid styles. Haematoxylin in any form gives better staining than cotton blue; and Flemming gives a better fixation than the alcohol-acetic-lactic mixture as a preliminary to haematoxylin.

#### PART I. *PRUNUS CANTABRIGIENSIS* AS FEMALE PARENT

Pollinations were made with (1) itself, (2) 10/34, (3) Windsor (Sweet), (4) 9/34 (triploid), and (5) Duke  $\times$  Sweet (triploid). The observations, which are compatible or partially compatible, are shown in Tables 1 and 2, together with the fertility numbers of the respective pollinations and the average growth rate of their pollen tubes. The style, which is 7–8 mm. long, was divided for purposes of classification into four arbitrary sections. Thus the lengths were grouped in four classes of about 2 mm. each, with a fifth class of grains which failed to germinate.

##### *Prunus cantabrigiensis* selfed

Nearly all pollen tubes travel the full length of the styles and are competent to fertilize the ovules. A percentage of 41 fruits set and matured, which is high for a cherry. Only a few grains remained ungerminated on the stigmatic surface; their failure is presumably due to inherent sterility. The distribution of the ends of pollen tubes 4 days after germination is better illustrated by Text-fig. 1, which is adapted from a type of diagram used by Buchholz & Blakeslee in the case of *Datura* (1929). In this and the subsequent diagrams the horizontal line repre-



Text-fig. 1. *Prunus cantabrigiensis* selfed. Four days.

sents the length of the style, the thick vertical line on the left is the percentage of ungerminated pollen grains found on the stigma, and the

Table 1. Pollinations with pollen of diploids and tetraploids

Pollination	Av. length of style in mm.	Length of sections in mm.	No. of pollen tubes in				Growth rate mm./day	Fertility	
			2 days	4 days	6 days	8 days		Flowers pollinated	Fruits matured
<i>P. cantabrigiensis</i> (tetraploid) selfed	7.4	0	2	2	—	—	1.0	368	41
		0.1-1.9	7	2	—	—			
		2.0-3.9	10	1	—	—			
		4.0-5.0	—	15	5	2			
		6.0-7.4	—	21	2	—			
<i>P. cantabrigiensis</i> × 10/34 (4x)	7.3	0	—	—	—	—	2.7	129	41
		0.1-1.9	33	33	27	29			
		2.0-3.9	39	20	7	5			
		4.0-5.9	6	2	1	2			
		6.0-7.3	6	17	16	4			
<i>P. cantabrigiensis</i> × <i>P. avium</i> (Windsor) 2x	7.3	0	5	3	4	2	1.5	151	29
		0.1-1.9	46	24	14	26			
		2.0-3.9	14	25	1	—			
		4.0-5.9	—	53	1	—			
		6.0-7.3	—	1	5	2			
10/34 (4x hybrid) selfed	8.4	0	18	23	—	—	—	37	21
		0.1-1.9	4	19	—	—			
		2.0-3.9	2	5	—	—			
		4.0-5.9	—	7	—	—			
		6.0-8.4	—	2	—	—			
<i>P. avium</i> (Baumann's May) 2x × <i>P. cantabrigiensis</i>	12.4	0	40	9	—	—	1.4	101	29
		0.1-1.9	51	13	—	—			
		2.0-3.9	5	11	—	—			
		4.0-5.9	—	3	—	—			
		6.0-7.0	46	32	16	—	0.7	—	—
<i>P. avium</i> (Baumann's May) 2x × 10/34 (4x)	12.6	0	7	7	3	—			
		0.1-1.9	3	2	3	—			
		2.0-3.9	1	1	—	—			
		4.0-5.9	—	—	—	—			
		6.0-7.0	—	—	1	—			

Table 2. *Pollinations with pollen of triploids*

Pollination	Av. length of style in mm.	3 days		6 days		Length in mm.	Fertility	
		No.	Length in mm.	No.	Length in mm.		Flowers pollinated	% Fruits matured
<i>P. cantabrigiensis</i> 4x × 9/34 ( <i>P.</i> <i>cantabrigiensis</i> × <i>P. avium</i> )	7.3	1	0.2	1	0.2	7.4	293	9.5
<i>P. cantabrigiensis</i> × (Sweet × Duke)	7.9	1	0.5	1	0.5	1.0	71	4.2
<i>P. avium</i> (Baumann's May) 2x × (Duke × Sweet)	12.8	1	0.9	1	2.3	8.0	643	2
<i>P. avium</i> (Baumann's May) 2x × (Sour × Sweet)	12.2	—	—	1	1.1	—	76	0
<i>P. avium</i> (Baumann's May) × ( <i>P.</i> <i>cantabrigiensis</i> × <i>P. avium</i> )	13.2	1	0.5	1	3.7	—	59	0

remaining numbers are the percentage frequencies of the pollen tubes at successive intervals of 1 mm. length. The upper part of the horizontal line is devoted to apparently normal pollen tubes, the lower part to those showing marked signs of incompatibility. It was found that compatible pollen tubes in cherries grow down the style without any change of their original shape on penetration, apart from a slight thinning; but incompatible tubes may cease to grow at any point, the ends of such tubes becoming bent or swollen (Pl. 4, figs. 1, 2; Afify, 1933; Roy, 1938). Burst pollen tubes, common in *Datura*, are rare in the cherries.

Four days after pollination the modal class of tubes is near the micropylar region. At this time two tubes were seen in the ovule chamber. At the sixth and eighth day after pollination no pollen tubes were observed near the stigma and only a few near the micropyle (Table 1).

*Prunus cantabrigiensis* × 10/34 (*P. cantabrigiensis* × *P. cerasus*)

The factorial theory of incompatibility, which works well in diploid species, is naturally complicated in the case of polyploid hybrids. This complex behaviour is shown by the present pollination. As we see from Table 1, 40 % of the pollen grains fail to germinate on the stigma, and the true proportion of ungerminated grains is probably higher still, as some are inevitably lost in the process of fixation. In these counts only the medium and large grains are included; of these 75 % are germinable on artificial media (Raptopoulos, 1940). We have therefore a failure to germinate of at least 15 % above the 25 % which do not germinate in artificial media.

In this and in other pollinations examined the effective pollen grains do not germinate simultaneously on the stigma, but at successive intervals. This time differentiation is probably due to the differential absorption of the stigma secretion, owing to crowding. Such competition for the stigma secretion may account for part of the ungerminated pollen grains, though genetical reasons inhibiting their germination cannot be entirely excluded.

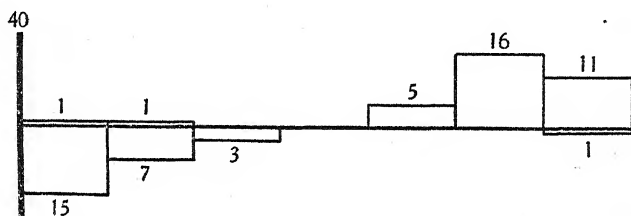
The pollination can be considered as a *partially compatible* one.<sup>1</sup> By the second day some compatible tubes have already reached the ovaries, while at the same time a few tubes near the stigma have swollen or bent ends. Four days after pollination all the compatible tubes are near the ovary, while another group of penetrating tubes shows incapacity for

<sup>1</sup> Where partially compatible pollination means that only a portion of the pollen tubes effect fertilization, the rest being checked in the style.

further growth (Text-fig. 2). At this time the pollen-tube ends cover the whole length of the style with a bimodal distribution: one modal class of incompatible tubes just below the stigmatic surface, the other of effective tubes near the ovary. Most of the incompatible tubes near the stigmatic area had their ends bent upwards, the others were mostly swollen.

Further examination of the growth after the sixth and eighth day shows that while the actual number of pollen tubes decreases considerably the two groups still exist, but only the incompatible tubes are visible, the compatible ones having disappeared into the ovary chamber.

The peculiar behaviour of the present pollination cannot be explained on a monofactorial hypothesis. Several factors for incompatibility are



Text-fig. 2. *Prunus cantabrigiensis*  $\times$   $F_1$  10/34. Four days.

involved which are responsible for the behaviour of the pollen tubes. The following groups appear genotypically controlled:

- (1) Compatible tubes which reach the ovary.
- (2) Incompatible tubes which end near the stigmatic surface.
- (3) Incompatible tubes which end in the lower half of the style.

It is probable that the second and third groups differ only in the number of incompatibility factors present.

#### *Prunus cantabrigiensis* $\times$ *Windsor* (Sweet)

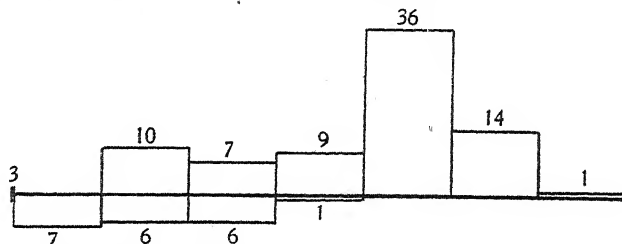
This pollination is quite different from the other two as regards the distribution of the pollen tubes inside the stylar tissue.

Two days after pollination the foremost pollen tube scarcely reaches the middle of the style (Table 1). At the same time the first incompatible pollen tubes appear near the stigma.

The haploid pollen can effect fertilization only after 4 days, when the first pollen tubes reach the ovary, but the pollination is effective and 23 % of the flowers pollinated set and mature fruits. At 4 days there is only one modal class (Text-fig. 3) of compatible pollen tubes in the lower half of the style. The other compatible pollen tubes have a great

range of variation, stretching from the ovary up to a distance of 1 mm. from the stigma. Of the total tubes germinated 20 % show clear signs of incompatibility and stop growing.

Six days after pollination the main group of pollen tubes remaining in the style are the incompatible ones, with a few compatible tubes still scattered at different levels and proceeding slowly to the micropyle. Eight days after pollination only incompatible tubes are found lying in the upper half of the style, and near the stigmatic surface. In *P. cantabrigiensis* the styles have poorly developed intercellular spaces, which in some crosses appears to restrict the growth of the tubes mechanically. In several styles, for example, it was observed that one or two pollen tubes instead of developing regularly had an acute end behind which lay a swollen part such as would ordinarily be considered a mark of incompatibility. In other cases this swollen part lay farther back from the



Text-fig. 3. *Prunus cantabrigiensis* × Windsor. Four days.

point and probably represents a temporary check to growth from which the tube afterwards recovered by a change of direction. On one occasion a forked pollen tube was found (Pl. 4, fig. 4); the tube afterwards recovered by a change of direction. On one occasion a forked pollen tube was found (Pl. 4, fig. 4), which may be due to the same cause. Forked pollen tubes in artificial media have been reported by Crane & Thomas (1939) in pears. Silow (1931) reports that many pollen tubes in red clover styles stop at a certain distance from the stigma, presumably from a mechanical obstacle in their way, because this phenomenon affects compatible and incompatible tubes equally. Similar abnormalities found in *Datura* by Buchholz & Blakeslee (1929) may have the same origin.

The results of pollinating *P. cantabrigiensis* with the diploid *P. avium* "Windsor" agree with observations made on the diploid cherries, namely, that in every partially compatible cross-pollination with pollen of diploids, two genotypes occur:

- (1) with complete compatibility,
- (2) with complete incompatibility.



*Prunus cantabrigiensis* × pollen of two triploids

The triploids 9/34 (*P. cantabrigiensis* × Sweet Cherry) and Sweet (Bohemian Black) × Duke (Reine Hortense) were the male parents in these pollinations. The proportion of good pollen is very low; about 5 % looks good, but only 1 % germinates on artificial media. Those grains which germinate take almost twice as long to form their tubes as do the diploids and tetraploids.

Table 2 gives the results of pollinations along with percentage of fruit set. Intervals of 3, 6 and 9 days were chosen in view of the slow growth rate. Abundant pollen was laid on the stigma to counteract the high sterility. In spite of this only a few grains germinated, and very few penetrated the stigma to any depth. In each case some apparently normal pollen grains, deeply stained, lay on the stigma without any sign of germination. The majority of the tubes are short, and bent or swollen, but two tubes, one in each pollination, succeeded after 9 days in reaching the ovary chamber and effecting fertilization. From the cross with the pollen of 9/34 four seedlings were secured, two of which were found from root-tip counts to be tetraploids. Two seeds of the pollination with the second triploid hybrid failed to germinate. It is possible that the pollen tube had penetrated deeply enough to promote a parthenocarpic development of the embryo-sac, or what is more probable, a zygote was formed but died shortly after fertilization.

From cytological and breeding observations it is likely that the tubes which travelled the whole length of the style had an orthoploid constitution. It is not certain whether the short pollen tubes found in these pollinations are due to incompatibility factors or aneuploid constitution.

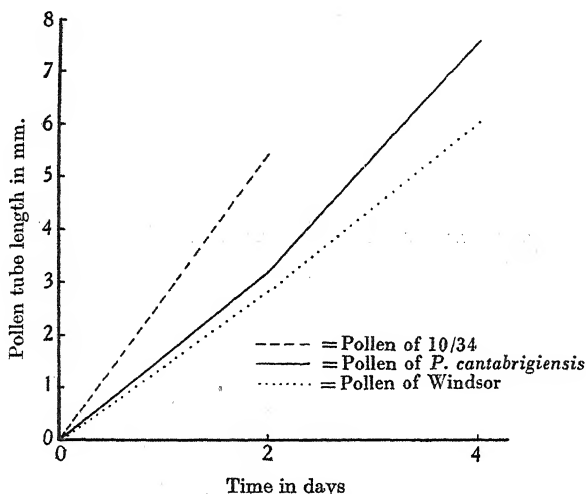
We may note the relative success of the pollinations with pollen of triploids, which in the case of 9/34 gave 9 % fruits. In these artificial pollinations, where care is taken to provide an even and plentiful supply of pollen, good results may be obtained even if very little of the pollen is viable. Under natural conditions, of course, the chance of success would be much lower.

*Growth rate.* In calculating the growth rate of the different pollen tubes inside the style of *P. cantabrigiensis* I have not taken into account those of the triploids, because of their small number. The first three pollinations, that is, the selfing and crossing with 10/34 and Windsor, were made on the same day under strictly comparable conditions. Since the number of pollen tubes entering the style is comparatively small, the

average daily increase in length of the three foremost compatible pollen tubes was chosen and will be referred to as the *growth rate* of the sample. These pollen tubes, of course, must be recorded before reaching the ovary, consequently only 2-4 days observations are available for the purpose.

The average distance travelled by the three foremost pollen tubes is given in Text-fig. 4. By the use of 2-day intervals the effect of temperature can be partially overcome.

We see from the diagram that the pollen tubes of 10/34 have the highest growth rate, equal to 2.7 mm./day (Table 1). The pollen tubes from the diploid have the lowest rate (1.5 mm./day). Those of *P. cantabrigiensis* are intermediate, 1.9 mm./day.



Text-fig. 4. Average pollen tube growth. Mother plant: *Prunus cantabrigiensis*.

These observations explain why, when pollen of triploids was put on the tetraploid style, only the  $2n$  pollen grains effected fertilization, competing successfully with the haploid grains, which according to the cytological observations are formed in equal numbers. The style of the tetraploid Chinese Early offers an easier path to the pollen of tetraploid cherries, restricting the growth of the haploid pollen to such a degree that it is unable to compete with the diploid pollen, though the haploid by itself may act as a good pollenizer. As, in styles of *P. cantabrigiensis*, pollen of 10/34 grows faster than its own pollen, mixed pollinations should favour cross-fertilization.

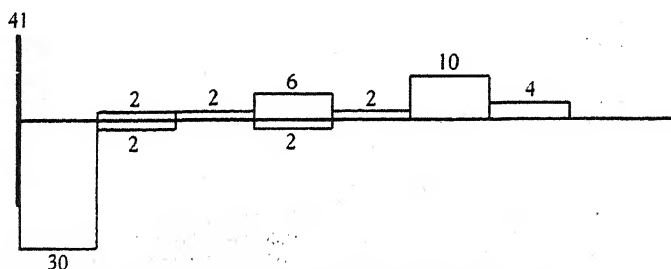
The type of curve followed by the pollen tubes on self-pollination and cross-pollination with Windsor is similar to that found by East & Park

for *Nicotiana* (1918). The pollen growth accelerates on the third and fourth days. Unfortunately, we have no observations at shorter intervals to find out if the lower rate on the first and second day is due to loss of time during germination, as reported by Buchholz & Blakeslee in *Datura* (1927), and Emerson in *Oenothera* (1938).

PART II. SELF-POLLINATION OF 10/34 (*PRUNUS*  
*CANTABRIGIENSIS* × SOUR CHERRY)

The pollinations of this tetraploid hybrid were made out of doors in 1939. Its fertility was tested during 1940 in the greenhouse.

The results from both pollinations are given in Table 1. The temperature during these outdoor pollinations was uncontrolled and quite low. This is probably the reason why 2 days after pollination only six pollen grains have penetrated the style. Four days after pollination the



Text-fig. 5.  $F_1$  hybrid 10/34 selfed. Four days.

first compatible pollen tubes reach the micropylar region. By that time 30 % of the pollen grains have formed a short tube and have ceased to grow (Text-fig. 5). The tube is usually bent and not more than 0.1 mm. long; a few grow away farther, but even these do not reach half the length of the style. The compatible tubes are distributed throughout the style, with the majority in the lower half. Of the flowers pollinated during 1940, 21 % set and matured fruits.

The percentage of ungerminated pollen grains found on the stigma is exactly the same as that when the same pollen is used on *P. cantabrigiensis*, which is probably due to pollen sterility. Certain pollen grains, however, even 4 days after pollination had developed a very short tube which was unable to penetrate the stigma. No such effect was seen when the same pollen was grown in an artificial medium. The cause may lie in the low temperature then prevailing, or in a reaction, similar to that offered by the stylar tissue, with the stigmatic surface.

The compatible pollen tubes had a rate of 0.9 mm./day for the first 2 days and 1.5 mm. for the second 2 days. The acceleration cannot be explained by a rise in temperature, but only by a delayed germination which greatly decreased the rate of growth during the first 2 days.

The behaviour of this partially compatible pollination, with the large number of ineffective tubes and the widely spread incompatible ones, suggests the presence of at least two genotypes, compatible and incompatible. Here more data are needed to decide whether there is a third genotype.

#### *Self-pollination of two triploids*

Observations were made 3, 6 and 9 days after pollination. In spite of the large quantity of pollen used in pollinating the triploids 9/34 (*P. cantabrigiensis* × Sweet Cherry) and Sour × Sweet Cherry the pollen tubes which penetrated the style were very few. Normal-looking, ungerminated grains were found on every style, varying from three to four, but only four pollen tubes were observed entering the styles of 9/34 and two those of Duke × Sweet on the sixth day. These figures are even lower than when the same triploids were used to pollinate the *P. cantabrigiensis* style.

The pollen grains which germinated and penetrated the style had a very short tube, under 1 mm. even after 9 days, their growth rate is so slow that it is highly improbable they would reach the ovary during the lifetime of the flower. Furthermore, two of the ends of the 9/34 pollen had clear signs of incompatibility. It is evident that incompatibility is here enhanced by the effect of mechanical restrictions to growth, as described above.

### PART III. POLLINATIONS WITH *PRUNUS AVIUM* AS FEMALE PARENT

In the pollinations carried out with the diploid cherry Baumann's May only pollen of tetraploid and triploid cherries was used, since the behaviour of the self-pollinations has been described by other authors (Roy, 1938). Tables 1 and 2 give the results obtained, together with growth rate of pollen and fertility numbers.

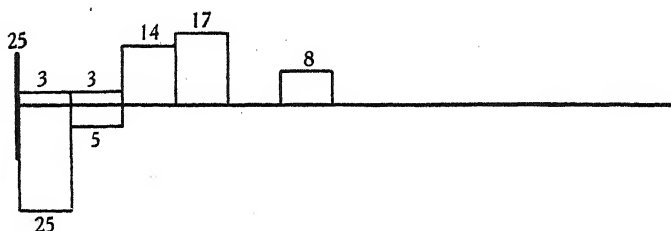
#### *Baumann's May* × *Prunus cantabrigiensis*

Two days after pollination a large proportion of the pollen (38 %) was found on the stigma unable to penetrate the style. By that time many pollen grains had developed a short pollen tube, but they had not entered the style. Most of the pollen tubes which did penetrate had signs of

incompatibility. A few, however, succeeded in reaching a depth of 2.9 mm. The percentage distribution 4 days after pollination is given in Text-fig. 6. From this and from Table 1 we see that 25 % of the pollen still rests ungerminated on the stigma.

The high proportion of ungerminated pollen grains cannot be explained entirely on the ground of inherent sterility, as the same pollen had a higher germination on its own stigma and in artificial media.

Almost half of the tubes that entered the style stopped near the stigma. Of the apparently compatible tubes only three succeeded in reaching a depth of 5 mm., with their foremost tube at a distance of



Text-fig. 6. Baumann's May  $\times$  *Prunus cantabrigiensis*. Four days.

5.7 mm. from the stigma, which is less than half the length of the style. This behaviour of  $2n$  pollen on the  $2n$  style is quite different from that of the reciprocal cross, when the haploid ( $n$ ) pollen had travelled in 4 days the whole length of the style of *P. cantabrigiensis*, with two pollen tubes inside the ovary chamber. It explains the really considerable difference between these two reciprocal pollinations in the number of fruits set and matured.

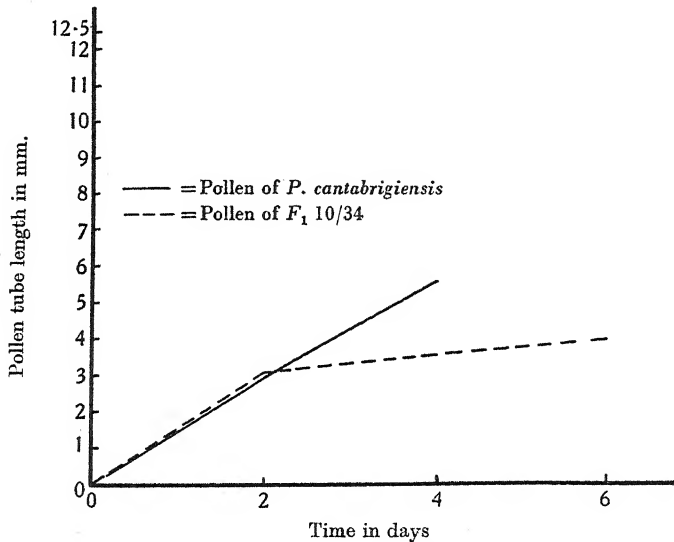
#### *Baumann's May $\times$ $F_1$ hybrid 10/34*

The behaviour of the pollen of this tetraploid on the diploid style is very similar to that described above. Few if any tubes reach the ovules.

*Growth rate.* The average growth rate of the pollen tubes of *P. cantabrigiensis* and 10/34 calculated at 4 and 6 days after pollination is much lower than when the tetraploid species is used as female (Table 1). At the rate of 1.4 mm./day the pollen tube of *P. cantabrigiensis* would need 9 days to travel the length of the diploid style, and the hybrid 10/34 would need twice this time. This is assuming the growth rate to be constant; actually it appears to fall off (Text-fig. 7), which decreases still further the effectiveness of the pollination. Analogous curves are reported by Silow (1931) in clover.

*Baumann's May crossed by three triploids*

Very few pollen tubes enter the style, and very few fruits set in spite of the large number of flowers pollinated. The results are set out in Table 2. The stigmatic surface was covered with aborted pollen grains and normal-looking grains which failed to germinate. The few grains



Text-fig. 7. Average pollen tube growth. Mother plant: Baumann's May ( $2n$ ).

which germinated formed a thick tube which did not travel more than 3.7 mm. even in 6 days. It is therefore improbable that the tubes could finish the journey in time to bring about fertilization.

The two seeds obtained from the first pollination had no endosperm, only an empty testa.

When we compare Tables 1 and 2 we see that the same pollen had a different action on the styles of the tetraploid and diploid cherries, with more favourable results in the former case.

## DISCUSSION

The behaviour of the diploid cherries is relatively simple, that of the polyploids more complex, as the greater number of allelomorphs present leads to a greater number of possible combinations.

*P. cantabrigiensis* selfed was found to be fully compatible; all other pollinations, with the exception of the triploids selfed, were partially

compatible. In each case a number of pollen tubes travelled the whole length of the style and effected fertilization, while others became arrested in their growth and developed swollen or bent ends.

The part played by the stigma calls for some notice. In *Trifolium pratense*, according to Martin (1913), it acts only as a regulator of water supply to the pollen, and the arrest of incompatible pollen tubes only takes place after the exhaustion of their initial food supply (Silow, 1931). In contrast to this passive behaviour, the style in the cherry seems to offer resistance to the pollen carrying the same incompatibility factors, from its very germination. This reaction may act at any time from germination until the tubes reach the micropyle. This behaviour is very clear in the case of *P. cantabrigiensis*  $\times$  10/34, where 8 days after pollination incompatible pollen tubes were found throughout almost the whole length of the style.

While in the cross-pollinations the results of pollen-tube growth agree to some extent with the oppositional hypothesis, two self-pollinations, those of 10/34 and *P. cantabrigiensis*, fail to conform with the original explanation. As we have seen, *P. cantabrigiensis* selfed is wholly compatible, and only a part of the pollen tubes of 10/34 are arrested in its own style. It is probable that these plants possess one or more factors for self-fertility.

We are now in a position to answer the questions at the beginning of this section. The growth rate of the pollen tubes is an essential factor for the success of the pollination. If the compatible pollen tubes are able to reach the ovary during the lifetime of the flower, then the pollination is effective.

The fertility figures obtained from these pollinations fully agree with the behaviour of the respective pollen. As we have seen, when *P. cantabrigiensis* is crossed with its own pollen and the pollen of 10/34 the percentage of fruits is high (41 %). This number falls to 29 when pollen of diploid cherries is used. But the fertility goes down to 3 % when the diploid style is pollinated by the pollen of tetraploids.

Thus when a mixture of pollen of diploids and tetraploids is used the style will offer more favourable conditions to the pollen of the tetraploids, although a proportion of diploids will be successful. Further, the almost complete failure of the pollinations when the diploid is used as female indicate that to secure cross-pollinations the cross should always be made with the tetraploid as the female, not the other way about.

No differences were found between reciprocal pollinations in the diploid cherries (Crane & Lawrence, 1934), but when cherries with

different chromosome numbers are crossed, certain aberrations occur among the results of the pollinations.

Whether Sirks's (1926) explanation of reciprocal pollinations, based on a modification of the incompatibility hypothesis, is maintained or not, several other factors seem involved in the case of the present material.

As we have seen earlier, the styles of *P. cantabrigiensis* and 10/34 average 7.6 and 8.4 mm. respectively, which is much shorter than the length in the diploid cherry (12.6 mm.). It is possible therefore that, as in *Nicotiana*, the pollen tubes of the two short-styled cherries are not adapted to travel the extra length of the diploid style and so are unable to bring about fertilization. When the sweet cherry is used as male parent the discrepancy is in its favour, and the tube is fully able to reach the ovary. It is worth noting that the tubes of *P. cantabrigiensis* in the style of Windsor in 4 days travelled 6 mm. from the stigma, a distance roughly equal to the whole length of its own style. Support for this explanation comes from the fact that when almost equal-styled cherries are crossed (Dukes  $\times$  *avium*) the difference between reciprocal pollinations is not great.

Apart from the difference in style length, the plants concerned differ also in chromosome number. It has been observed that, as in the case of *Campanula*, *Triticum*, *Galeopsis*, etc., the pollination is more successful when the higher numbered plant is used as the female. Roy (1938) pollinated the hexaploid *Prunus domestica* with the diploid *P. divaricata* as male, and reported better results in this direction than in the reciprocal cross.

From the behaviour of the pollinations described it is clear that intercrossing between the diploid and tetraploid species examined is greatly restricted, and consequently the chance of triploids being formed by natural cross-pollination is very small.

#### SUMMARY

Studies of pollen-tube growth on diploid, triploid and tetraploid styles with the same range of pollen have shown:

1. The tetraploid cherry *Prunus cantabrigiensis* is self-compatible.
2. Self-pollination of triploids fails entirely, in part because of the high sterility of the pollen and in part from the deformation of the styles and the presence of opposing factors for incompatibility.
3. The style as well as the stigma takes an active part in resisting incompatible pollen tubes.



4. Almost all the cross-pollinations examined are partially compatible, but the proportion of effective pollen tubes ranges considerably.

5. The growth rate of the compatible pollen tubes accelerates on the third and fourth day after pollination, whilst that of the incompatible tubes is greatly decreased and in many cases may be completely arrested in the stylar tissue at this time.

6. Cross-pollinations between diploids and tetraploids are favoured when the tetraploid is used as the female parent.

7. The complexity in behaviour increases with polyploidy and hybridity.

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Fig. 1.

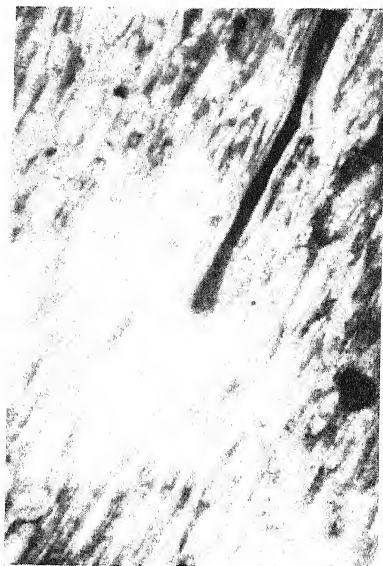


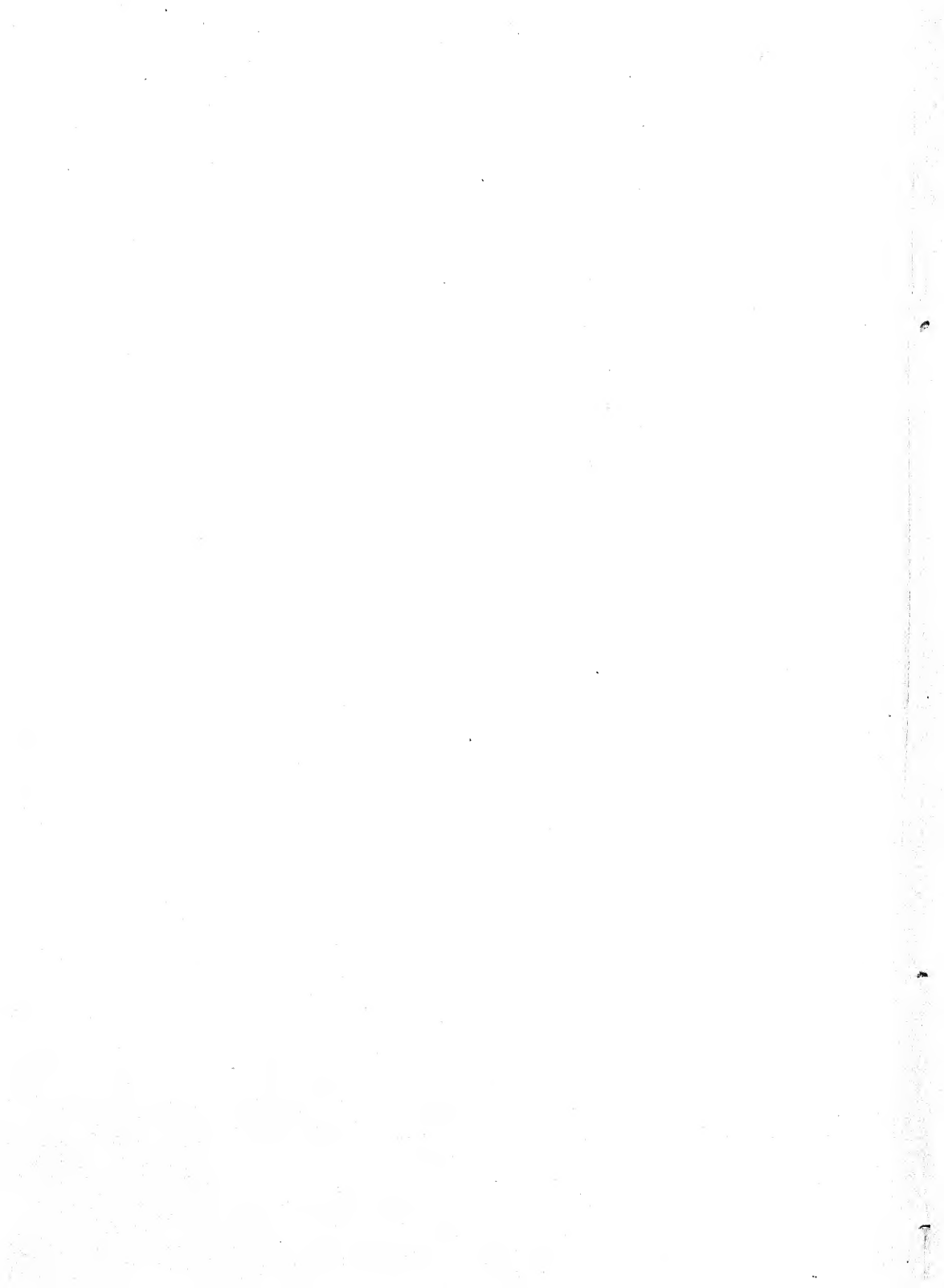
Fig. 2.



Fig. 3.



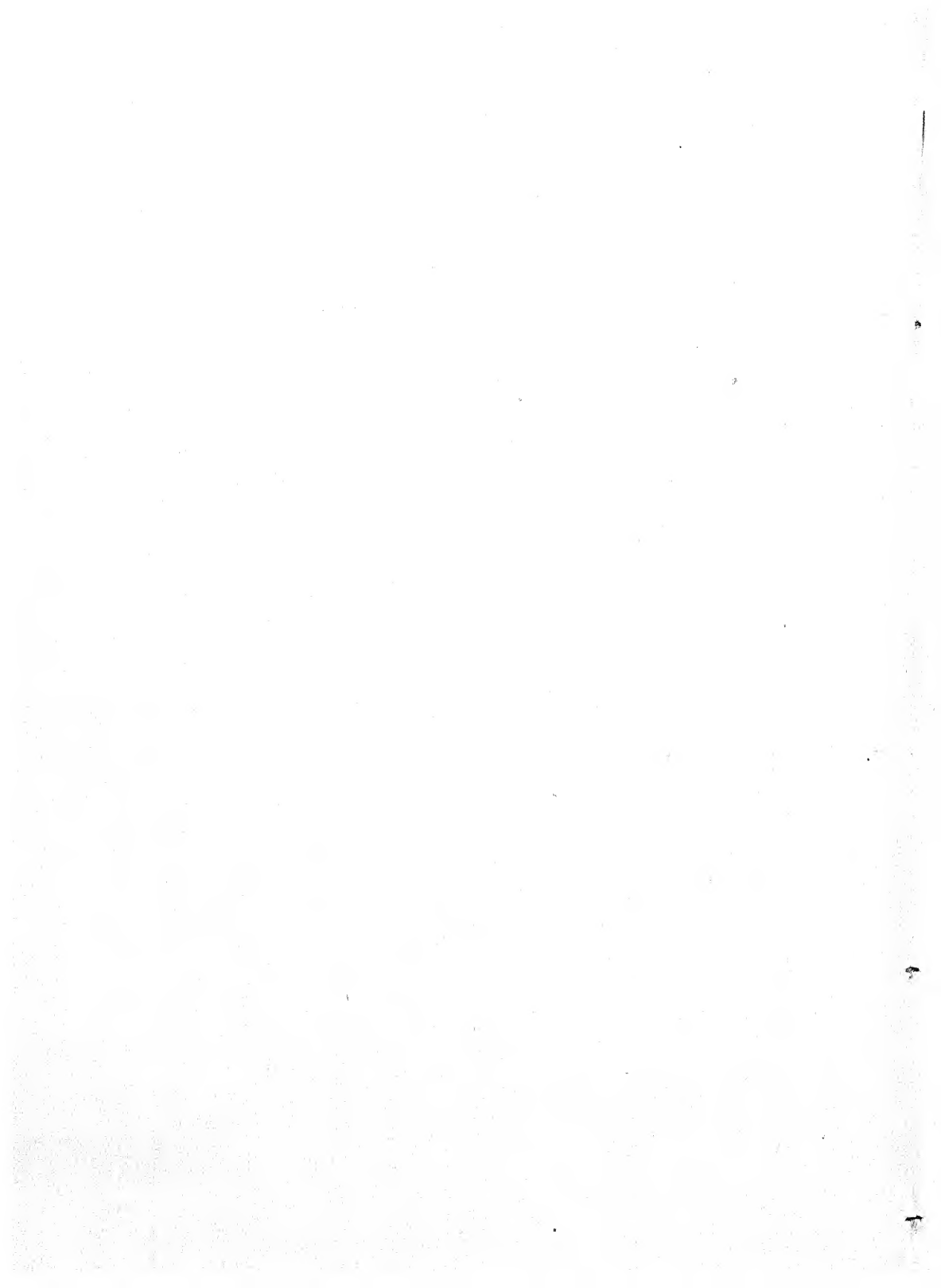
Fig. 4.



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## EXPLANATION OF PLATE 4

- Fig. 1. Compatible and incompatible pollen tubes in the cross *P. cantabrigiensis*  $\times$  10/34. 2 days, 0.6 and 0.2 mm. respectively.  $\times$  80.
- Fig. 2. Incompatible pollen tube in the partially compatible cross *P. cantabrigiensis*  $\times$  Windsor. 8 days, 1 mm.  $\times$  400.
- Fig. 3. The two gametic nuclei in a pollen tube of the cross Baumann's May  $\times$  *P. cantabrigiensis*. 2 days.  $\times$  360.
- Fig. 4. Forked pollen tube inside stylar tissue of pollination *P. cantabrigiensis*  $\times$  Windsor. 4 days.  $\times$  360.



# CHROMOSOMES AND FERTILITY OF CHERRIES AND THEIR HYBRIDS<sup>1</sup>

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(With Plates 5 and 6 and Twelve Text-figures)

## INTRODUCTION

ALTHOUGH much information is available on the cytology of the species and varieties of cultivated cherries, no full comparative account of their chromosome behaviour has yet appeared. Chromosome differentiation in this section of *Prunus* is not pronounced, but a study of the species and of various new hybrids gives us a clearer view of their interrelations.

Results of detailed cytological study of cherries and related *Prunus* species are presented in this paper. The new conclusions reached on the origin and constitution of cultivated cherries will be discussed in relation to our present knowledge of the taxonomy and genetics of the group. Special attention has been paid to quantitative data on the metaphase configurations, and care has been taken to avoid selection which would affect the data (Mather, 1939).

## MATERIAL

The material consisted of the following plants, all falling under the subgenus *Cerasus*:

Diploid species, $2n=16$	<i>P. avium</i>
Tetraploid species, $2n=32$	<i>P. cantabrigiensis</i> *
	<i>P. cerasus</i>
	<i>P. fruticosa</i>
	Duke Cherries
Tetraploid hybrids	$F_1$ <i>P. cantabrigiensis</i> × <i>P. cerasus</i>
	Duke × Sour Cherry
Natural and artificial triploids, $2n=24$	$F_1$ <i>P. cantabrigiensis</i> × <i>P. avium</i>
	$F_1$ <i>P. cerasus</i> × <i>P. avium</i>
	$F_1$ Dukes × <i>P. avium</i>
	<i>P. avium nana</i>
	<i>P. Lannesiana</i> †

\* *P. cantabrigiensis* Stapf = *P. pseudocerasus* Lindl.

† *P. Lannesiana* Wilson = *Cerasus Lannesiana* Carr.

*P. avium nana* was kindly supplied by the Director of the Royal Botanic Gardens, Kew. All the other material studied was grown at Merton.

<sup>1</sup> Part II of a thesis approved for the Degree of Ph.D. in the University of London.

## METHODS

Both root tips and pollen mother cells were examined. Root tips were fixed in La Cour's 2 BE (La Cour, 1937) and medium Flemming, and after dehydrating and embedding in the usual way were cut at 4-6  $\mu$ , and stained by Newton's gentian violet-iodine method.

After several tests to discover the correct stage of meiosis, well dissected buds were fixed in 3/1 absolute alcohol/acetic acid (McClintock, 1929) and transferred after 24-48 hr. to 70 % alcohol for storage. The pollen mother cells were stained with Belling's (1926) iron acetocarmine, the solution being modified to meet the needs of different plants. The modification (Thomas, 1940) consisted of diluting the carmine with 45 % acetic acid and adding varying proportions of iron acetate or chlorate. It proved very successful for this otherwise difficult material, particularly at diakinesis and later stages; to get good staining at prophase is more difficult. Slight pressure on the cover-slip, combined with warming the slide, gives a better spacing of the chromosomes and increased differentiation in staining.

Most of the photographs and drawings were made from temporary slides. Permanent slides were prepared by a combination of the methods of Buck (1936) and McClintock (1929) perfected by La Cour.

Drawings were made at a magnification of 4250 and reduced to 1400; photos at 900, enlarged to 1800.

I am indebted to Mr M. B. Crane for the use of his material and to Dr P. T. Thomas for his advice.

1. THE SWEET CHERRY, *PRUNUS AVIUM* L.

Four varieties of the diploid *P. avium* were examined, namely, Governor Wood, Bohemian Black, Monstrueuse de Mezel and Guigne d'Annonay. Since they differ little in cytology their behaviour will be described collectively.

In general, both meiotic divisions are normal. At diakinesis eight paired configurations, one of which is attached to the nucleolus, are always seen. Of the eight bivalents present at first metaphase, 4-7 are rods and 1-4 rings (Text-fig. 1). The chromosomes are joined by 1-3 chiasmata, usually subterminal. Terminal and interstitial chiasmata are less frequent. In a few cases two univalents are formed, apparently through failure of chiasmata at pachytene.

The first anaphase is occasionally disturbed by the differential separation of the bivalents; the rods, with terminal chiasmata, separate

before the rings owing to the greater resistance offered by a greater paired length. After a short interphase the second division follows a normal course. Apart from this differential separation, which in any case does not upset the meiotic process, no abnormality of importance was observed in the diploid cherries.



Text-fig. 1. First metaphase in the sweet cherry Guigne d'Annonay. Eight bivalents.

*Quantitative data* were obtained from the analysis of twenty-five pollen mother cells at metaphase. The results are summarized in Table 1, which gives the proportions of rod and ring bivalents, the number of total and terminal chiasmata in every group and the chiasma frequency.

Table 1

Type of bivalent	No.	Total Xta	Terminal Xta	$\frac{\text{Terminal}}{\text{Total}}$ Xta	Xma frequency
Open (rod)	136	136	38	0.28	1.34
Closed (ring)	64	133	45	0.34	
Total	200	269	83		

It will be seen from the table that about two-thirds are rod bivalents, joined by a single chiasma, the remaining third being rings joined by two or occasionally three chiasmata. More terminal chiasmata are found in the rings than in the open rods. This holds for all the *Prunus* species here examined, and indicates a higher degree of repulsion in the ring chromosome.

The chiasma frequency per bivalent is only 1.34. All chromosomes with a terminal or subterminal centromere fail to form more than one chiasma, while those with a median or submedian centromere form two and occasionally three chiasmata.

The regular meiotic process of the diploids results in the formation of a large proportion of normal tetrads. From 120 cells analysed, 112 were normal tetrads (93.3 %), four had five daughter cells (3.3 %) and three had six (2.5 %) and one eight (0.8 %).

## 2. THE DUKE CHERRIES

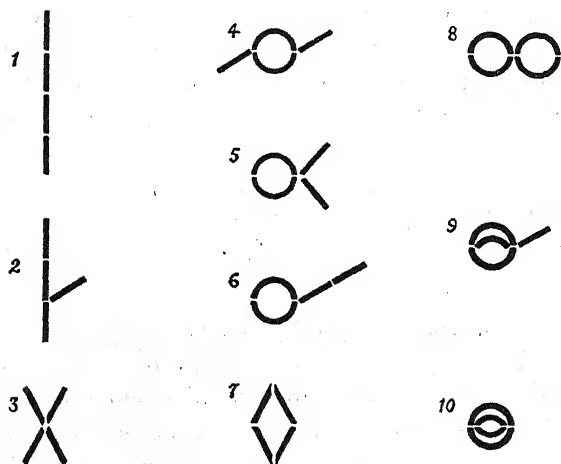
The Duke cherries are tetraploids ( $2n=32$ ). Their tetraploid constitution was found first by Kobel (1927); later Darlington (1928) and Hruby (1939) pointed out several peculiarities in their behaviour.



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Meiosis was examined in the following six varieties: Late Duke, Empress Eugenie, Royal Duke, Reine Hortense, Archduke, May Duke. These will be described together, only marked differences being mentioned.

At diakinesis eight to eleven paired configurations appear, but in the variety Late Duke as many as fourteen are occasionally found. At first metaphase univalents, bivalents, trivalents and quadrivalents may all be present. Univalents and trivalents usually correspond in number and thus indicate that they result either from failure to form enough chiasmata for a quadrivalent or failure to maintain enough chiasmata until metaphase. Usually one trivalent-univalent is present, but there may



Text-fig. 2. Ten possible quadrivalent associations with only terminal chiasmata.  
(After Darlington, 1931*a*.)

be two or none. If two univalents occur they may lie in the equatorial plate or out of it, and sometimes show a marked parallel relation one on each side of the plate.

The bivalents, either rods or rings, vary from 0 to 9. There are usually few bivalents, except in Late Duke which has an average of seven per metaphase plate. The bivalents separate and go to the poles first. The trivalents commonly form **V**, **Y** and **I** configurations.

Complete association, resulting in eight quadrivalents, was found in all the Duke varieties except Late Duke (Pl. 5, fig. 1). The quadrivalents are usually joined by terminal chiasmata. Eight out of the possible ten types (Darlington, 1931*a*) were observed, the commonest being 1, 2, 3, 5 and 7 (Text-fig. 2).

Anaphase is irregular owing to the presence of the univalents and multivalents. Univalents may divide in the first or second division, but no second split was observed either by direct observation or by counting the aggregates at the second telophase. The multivalents in their co-orientation fall into the types described by Darlington (1937). The significance of these types lies in their varying capacities for regular disjunction. A high proportion of indifferent and linear types of multivalent will lead to irregularities at anaphase. Lagging chromosomes are found to be common in both metaphases. As a result of univalent formation and unequal disjunction of multivalents the first telophase plates include unequal numbers of 15+17 or even 14+18, and also cases in which certain chromosomes lie outside both plates and form independent nuclei (microcytes).

The second division follows more normally. Syndiploidy is common in all Duke varieties, either complete (Archduke) or incomplete (Reine Hortense). Syndiploid cells are abnormally large, and there is a certain

Table 2

Configurations	I		II		III		IV		Xma fre- quency
	Total	Av. per P.M.C.	Total	Av. per P.M.C.	Total	Av. per P.M.C.	Total	Av. per P.M.C.	
Late Duke	13	0.65	141	7.05	15	0.75	76	3.75	1.51
Royal Duke	18	0.90	60	3.00	10	0.50	118	5.90	1.56
Empress Eugenie	14	0.70	66	3.30	10	0.50	116	5.80	1.61
May Duke	8	0.40	57	2.85	6	0.30	125	6.25	1.62
Reine Hortense	5	0.25	65	3.25	3	0.15	124	6.20	1.64
Archduke	8	0.40	54	2.70	8	0.40	125	6.25	1.66

difference in time of division between the chromosomes of the two nuclei.

The quantitative data in Duke cherries are based upon a complete analysis of twenty metaphase plates of each variety. Table 2 gives the total and average per metaphase of the various configurations and the chiasma frequency of different varieties.

An examination of Table 2 shows that Late Duke behaves differently from the other varieties. It forms more bivalents and fewer quadrivalents. Out of a total of eight possible quadrivalents, five varieties have an average of six and one variety of four, the variation being 1-8.

Table 3 gives a more detailed analysis of the metaphase configurations, with their frequencies, of the variety Reine Hortense, which may be regarded as typical of the last five varieties in Table 2 (i.e. of the group examined, excluding Late Duke). The pollen mother cells with eight and

seven quadrivalents number almost 50 % of the total analysed. Late Duke alone shows a wider range. The chiasma frequency (calculated as  $\frac{1}{2}$  chiasma per chromosome, cf. Darlington & Mather, 1932) appears in the last column of Table 2. Though fairly low (1.59), it is much higher than the corresponding figure for *P. avium* (1.34). The number of quadrivalents is related to the chiasma frequency; thus the variety Late Duke has the lowest chiasma frequency of the group.

The bivalents in Duke cherries show little variation in chiasma frequency. They form an average of about 1.3 half-chiasmata per chromosome, which is a little less than the frequency found in the diploid cherry. The real difference comes among the quadrivalents, which in the calculation increase the half-chiasmata to 1.6 per chromosome.

Table 3

Metaphase configurations	No. of P.M.C.
8 <sup>IV</sup>	3
7 <sup>IV</sup> 2 <sup>II</sup>	6
6 <sup>IV</sup> 4 <sup>II</sup>	4
5 <sup>IV</sup> 6 <sup>II</sup>	4
5 <sup>IV</sup> 1 <sup>III</sup> 4 <sup>II</sup> 1 <sup>I</sup>	1
5 <sup>IV</sup> 5 <sup>II</sup> 2 <sup>I</sup>	1
4 <sup>IV</sup> 2 <sup>III</sup> 4 <sup>II</sup> 2 <sup>I</sup>	1
	20

*Tetrad stage.* As the result of the abnormal meiotic behaviour and the occurrence of syndiploidy "tetrads" with a varying number of cells are found, the number varying from three to twelve with a modal class at the normal number of four. The groups from five to seven usually have four cells of about equal size and one, two or three micro-cells; the group above seven, which are the result of syndiploidy, have eight equal-sized cells and the rest micro-cells.

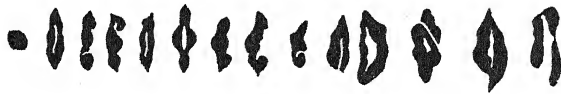
The number of normal tetrads may or may not have any relation with the pollen germination or fertility, as we shall see later, but here it does indicate the mechanical results of the uniformity of metaphase configurations. It was for instance found that the more uniform they are, the more normal the resulting tetrad, no matter whether the configurations are bivalent or quadrivalent.

### 3. THE SOUR CHERRY (*PRUNUS CERASUS*)

The tetraploidy of the sour cherries has been established since 1927 (Kobel, 1927; Darlington, 1928). In the present study the following varieties were examined cytologically: Morello, Wye Morello, Coe's Carnation and the closely related species *Prunus fruticosa*. As they differ little they will be discussed together.

Univalents, bivalents, trivalents and quadrivalents are found, but in proportions different from those in the Duke cherries (Pl. 5, fig. 2). Univalent and bivalent chromosomes are more frequent than in the Duke cherries. Trivalents are scarce. The main difference is found in the number of quadrivalents, the commonest type of which is a ring of four chromosomes. Coe's Carnation has more rings, Wye Morello and *P. fruticosa* more open types (Text-figs. 3, 4).

On account of the uni- and multivalents the first anaphase is rather irregular, and daughter telophase nuclei with thirteen and nineteen chromosomes have been observed; lagging univalents or division products of multivalents may form a continuous bridge of chromosomes



Text-fig. 3. Types of metaphase configurations in the sour cherries.



Text-fig. 4. First metaphase of *P. fruticosa*. Two quadrivalents, twelve bivalents.

between the groups in first telophase (Pl. 5, fig. 4). These as a rule fail to be included in the daughter nuclei and form separate groups. In certain cases whole quadrivalents were found lagging during disjunction (Pl. 6, fig. 1). As is usual in *Prunus* species, the univalents may divide in the course of either division. Syndiploidy, which is common in the Dukes, is here less common, though it does occur.

*Quantitative data.* The statistical observations are based upon the analysis of twelve to twenty pollen mother cells at the metaphase stage, as given below. Morello is omitted as only three pollen mother cells of this variety were examined. Table 4 gives the total of each type of configuration, its average per pollen mother cell and the chiasma frequency

Table 4

	No. of P.M.C.	I		II		III		IV		Xma frequency
		Total	Av.	Total	Av.	Total	Av.	Total	Av.	
Coe's carnation	20	39	1.95	190	9.50	15	0.75	44	2.20	1.30
Wye Morello	16	25	1.56	141	8.81	7	0.44	46	2.88	1.35
<i>Prunus fruticosa</i>	12	14	1.17	107	8.92	8	0.66	33	2.75	1.26

of the variety or species. The numbers show great similarity not only between the two sour cherries but also between them and *P. fruticosa*. As can be seen from the table, the number of univalents does not, as in the Dukes, correspond with the number of trivalents. The presence in so many cases of up to four univalents without trivalents indicates failure of pairing in the pachytene stage, not subsequent breakage of pachytene quadrivalents as in the Dukes. Twelve different combinations of configurations were observed in Wye Morello in sixteen pollen mother cells and eleven in Coe's Carnation in twenty pollen mother cells. Kobel (1927) and Darlington (1928) state that sixteen paired configurations may sometimes be seen in the sour cherries they examined. The present investigations show a continuous range from  $1^{IV} 14^{II}$  to  $4^{IV} 8^{II}$  within the sour cherry group.

*Chiasma frequency.* The chiasmata formed per paired configuration vary from 0 to 4, with an average frequency (Table 4, last column) for the sour cherries of 1.32, which is very close to that of *P. avium* (1.34).

*P. fruticosa*, a species examined for comparison with the cultivated sour cherries, proved very similar to them. Indeed in cytological behaviour it occupies a position intermediate between two varieties of *P. cerasus* examined.

*Tetrad stage.* Following abnormal behaviour and syndiploidy, from three to eight cells were found at this stage. The variety Coe's Carnation shows a greater abnormality in its tetrad stage, owing to the higher number of bivalents and trivalents.

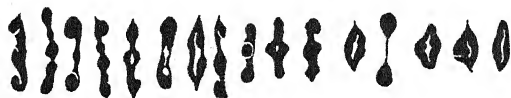
#### 4. *PRUNUS CANTABRIGIENSIS* (CHINESE EARLY)

*P. cantabrigiensis* Stapf (= *P. pseudocerasus* Lindl.) was found to be a tetraploid ( $2n = 32$ ). Two abnormalities were observed in early fixations, namely, (1) the pollen mother cells remained stuck together from early prophase to the tetrad stage, and (2) a higher chiasma frequency was found in 1940 than that recorded in 1939. Material fixed at a later date, however, showed neither abnormality and agreed with the 1939 findings. It is probable that the main cause of the change was the very low temperature prevailing during the time when the early meiotic stages were going on.

At pachytene full  $2 \times 2$  pairing was observed. Failure of pairing was common only on a small portion of a pair of chromosomes (Pl. 6, fig. 3), where it was presumably due to a small inversion. From early diakinesis sixteen paired configurations can be seen, three of which are attached

to the large nucleolus (Pl. 5, fig. 6). The number of nucleoli varies from one to six.

The first metaphase is regular and normal. Sixteen bivalents were found in 56 % of the metaphases examined (Text-fig. 5). No univalents or trivalents were found; in eleven pollen mother cells out of twenty-five analysed one quadrivalent occurred. In one only of fifty-two pollen mother cells examined two univalents were found, but these from their orientation appear due rather to precocious disjunction than a failure to pair. The bivalents are arranged at the metaphase plate in a narrow spindle, and the division is strikingly like that in the diploid cherry.



Text-fig. 5. First metaphase of *P. cantabrigiensis*. Types of bivalents.

The ratio of rod to ring bivalents is 2 to 1. Most chiasmata are sub-terminal, interstitial and terminal attachment being less frequent. The quadrivalents are usually of the ring type (Text-fig. 6), but a few other types are found. The chiasma frequency of the species (1.43) is higher than that of the sour cherries (1.30) but lower than that of the Dukes.

The only abnormality at anaphase comes from the presence of a quadrivalent in several plates. In most cases the quadrivalents separate regularly, with adjacent or alternate members going to the same pole.



Text-fig. 6. Types of quadrivalents found in *P. cantabrigiensis*.

Telophase is normal. At this stage four nucleoli usually appear, two large and two small. The second division is generally normal.

*Tetrad stage.* As a result of the normal meiotic behaviour we get normal tetrads. Of a total of 120 "tetrads" 96 % were four-celled. Such a degree of regularity has not been found in any other tetraploid cherry. It shows that *P. cantabrigiensis* is a functional diploid.

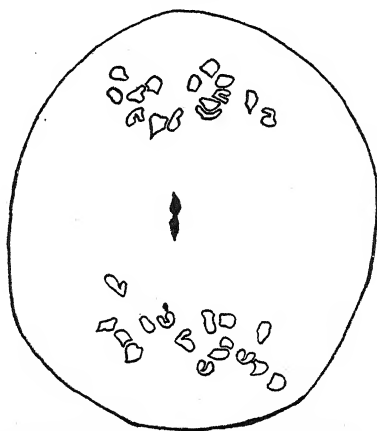
##### 5. THE TETRAPLOID HYBRIDS

The hybrids are (1) *P. cantabrigiensis* × *P. cerasus* (Coe's Carnation) (Seedlings 10/34), (2) May Duke (Duke) × Morello (sour). In the hybrid seedling 10/34 there is a striking variation in time of division of the

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pollen mother cells. In almost every flower pollen mother cells were found at every stage from prophase to tetrads. This phenomenon, which was also found in the triploid interspecific hybrids, is not very common among the diploid and tetraploid species. It occurred in the hybrid for two successive years, which is an indication that it is inherent in the hybrid and not a mere temperature effect.

At the first metaphase the paired chromosomes are mostly bivalents and quadrivalents (Pl. 5, fig. 5). With a very few exceptions every plate has one to two univalents, which may lag and divide between the two daughter first telophase groups (Text-fig. 7).



Text-fig. 7. First telophase in the hybrid 10/34. Univalent lagging and dividing on the equatorial plate.

The bivalents are usually rods, with a small number of rings. The trivalents agree in number with the univalents, and it is probable that as in the case of the Duke cherries they are broken pachytene quadrivalents. The one to four quadrivalents are usually rings, either parallel or discordant.

At anaphase a certain amount of abnormality is caused, not only by the univalents and trivalents but also by the quadrivalents, which may disjoin later than the bivalents. From one to five laggards were counted in several plates.

The second meiotic division is almost completely regular. Undivided univalents divide during the second division. Incomplete syndiploidy may occur, but it is very rare.

The  $F_1$  between Morello and May Duke shows very high quadrivalent formation compared with its parents (two to seven; usually four to six. All the Duke types are found (Pl. 6, fig. 2)). Another characteristic is that

if two univalents occur they show marked parallel association, lying one on each side of the plate. Only once were two univalents found on the same side. This phenomenon is more usual in the triploid hybrids. The whole meiotic process is more normal in this cross than in 10/34.

*Quantitative data.* The statistical observations are based on the analysis twenty nuclei of 10/34 and twenty-five of the Duke  $\times$  Morello hybrid. Table 5 gives the percentage frequency of quadrivalents. In

Table 5

	No. of quadrivalents							Total
	1	2	3	4	5	6	7	
<i>P. cantabrigiensis</i> $\times$ Coe's Carnation (10/34)	15	40	20	25	—	—	—	100
May Duke $\times$ Morello	—	4	8	4	32	24	28	100

both hybrids the variation is great, but the contrast in their metaphase configurations is clear. It is brought out even more in Table 6 where the total number and average per pollen mother cell of each configuration and the chiasma frequency of the hybrids is given. The difference between

Table 6

Cross	I		II		III		IV		Xma fre- quency
	Total	Av.	Total	Av.	Total	Av.	Total	Av.	
<i>P. cantabrigiensis</i> $\times$ Coe's Carnation (10/34)	16	0.80	216	10.80	12	0.60	39	1.95	1.34
May Duke $\times$ Morello	19	0.76	117	4.68	9	0.36	130	5.20	1.59

these two hybrids lies in the number of bivalents and quadrivalents present. The quadrivalents formed by the hybrid Duke  $\times$  Sour far exceed those found in the sour cherries and are almost at the level of the Dukes. This hybrid by its behaviour can be considered as a link between the Duke and Sour cherries. The hybrid 10/34 agrees in the number of multivalents with its *Cerasus* parent.

The chiasma frequency (Table 6 last column) of the hybrid Duke  $\times$  Sour is almost equal to the average found in the Duke cherries (1.6) and the pairing does not indicate hybridity. The chiasma frequency of 10/34 is 1.34 half-chiasmata per chromosome and so is intermediate between those of its parents (1.43, 1.30).

In species hybrids it is usual to find a decrease of chiasma frequency as compared with the parents (*Triticum*, Darlington, 1931b; *Nicotiana*, Goodspeed, 1934). In these cases, and particularly in the May Duke  $\times$  Morello cross, no decrease but actually an increase was observed. In both cases the number of quadrivalents reached the level of the higher parent.



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*Tetrad stage.* Four to nine cells were found in the tetrad stage from the analysis of 120 pollen mother cells. The proportion of normal tetrads in the hybrid Duke  $\times$  Sour corresponds with that in Duke cherries; 10/34 is more irregular. The occurrence of octads is the result of incomplete syndiploidy. In general the number of cells in the tetrads of the hybrids does not exceed that of the pure species.

### 6. THE TRIPLOID HYBRIDS

A study has been made of the following five types of triploids, of which the first three are of experimental origin and the last two of natural origin:

*P. cantabrigiensis*  $\times$  *P. avium* "Guigne d'Annonay" (9/34).

*P. avium*  $\times$  Duke and reciprocal (Bohemian Black  $\times$  Reine Hortense, Reine Hortense  $\times$  Governor Wood).

*P. cerasus* (Morello)  $\times$  *P. avium* (Mexel).

*P. avium nana*.

*P. Lannesiana*.

In the hybrid family 9/34 pollen mother cells at all stages were found together, as in 10/34. The prophases contained two paired configurations attached to the nucleolus. At first metaphase (Pl. 6, fig. 5) there were four to eight univalents, generally on the plate, but sometimes as many as three lying off it; as a rule eight paired configurations, equal numbers of rods and rings; trivalents usually three, occasionally four, which is apparently the upper limit. At telophase, bridges and lagging chromosomes form chains between the two daughter nuclei and render disjunction difficult. The first meiotic division sometimes fails almost from the beginning, and restitution nuclei are formed (Pl. 6, fig. 4). The second division gives only 31.7% apparently normal tetrads, and even some of these may be defective. There are 5% triads, form partial restitution, and 1.7% dyads, with genuine restitution nuclei. As many as eight products of division, of various sizes, may be formed, the most common being four and six (Pl. 6, fig. 3).

The other four triploids show the same types of degeneration, carried furthest in the natural forms. Pollen mother cells may degenerate before meiosis (Müntzing's "diplontic" as opposed to "haplontic" abortion). In *P. avium nana* whole anthers show diplontic abortion.

*Numerical data.* The statistical analysis of the metaphase configurations was based on twenty-five pollen mother cells for each of the three interspecific hybrids, ten of *P. avium nana* and six of *P. Lannesiana*.

Unfortunately the last two are insufficient for conclusive results, but they provide information on the behaviour of the species.

Table 7 gives the variation of every configuration, its frequency per pollen mother cell and the chiasma frequency of every species or cross.

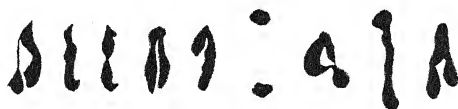
Table 7

	No. of P.M.C. analysed	I		II		III		Xma fre- quency
		Variation	Av.	Variation	Av.	Variation	Av.	
<i>P. cantabrigiensis</i> × <i>P. avium</i> , 9/34	25	4-8	6.32	4-8	5.84	0-4	2.00	1.15
Duke × <i>P. avium</i>	25	1-3	1.60	0-3	1.60	5-8	6.40	1.32
Sour × <i>P. avium</i>	25	1-5	2.92	2-5	3.28	3-7	4.84	1.30
<i>P. avium nana</i>	10	1-4	2.50	1-4	2.50	4-7	5.50	1.29
<i>P. Lannesiana</i>	6	1-4	2.00	1-5	3.50	4-7	5.00	1.22

As we see from the table, the hybrid 9/34 differs from the remaining four triploids both in the variation and the average of the configurations. It has many univalents and few trivalents, they have few univalents and many trivalents (Text-figs. 8, 9 and Pl. 6, fig. 6).



Text-fig. 8. First metaphase of Morello × Mezel. Five trivalents, three bivalents and three univalents.



Text-fig. 9. First metaphase of *P. avium nana*. Six trivalents, two bivalents and two univalents.

The hybrid 9/34 and *P. Lannesiana* have a chiasma frequency rather lower than the other three values, which lie close together. In spite of the low frequency in 9/34 there are usually eight paired configurations, indicating that two sets of chromosomes are capable of joining by chiasmata. The other four triploids, on the other hand, form chiasmata between all three sets. This behaviour is similar to that reported by Okabe (1928) in triploid *P. serrulata*. The high proportion of multivalents indicates autopolyploidy.

Three to seven different combinations of univalents, bivalents and trivalents are found on the metaphase plate. The variation of the

different combinations increases from the triploid Dukes  $\times$  *P. avium* to the hybrid 9/34, but with two exceptions, the sum of the paired configurations is always eight. Of the plates of the cross *P. cantabrigiensis*  $\times$  *P. avium* 16 % consist of seven paired configurations. Vaarama (1939) found similarly, in the case of the supposed allotetraploid *Rubus caesius* crossed by the diploid *R. idaeus*, that several metaphase plates of the triploid hybrid had less than seven paired configurations. The opposite effect is found in *P. Lannesiana* where 51 % of the metaphase plates have nine "paired" configurations. This pairing within the haploid set may also explain the parallel association of two univalents which is so common in the rest of the triploids—except 9/34. It is possible that they were at least partially paired but had failed to form chiasmata.

*The tetrad stage.* Except in the case of *P. avium nana*, the "tetrads" formed by all triploids are in accordance with expectation. *P. avium nana* has exceptional formation of supernumerary micro-cells, which does not seem to result directly from abnormalities of meiosis.

## DISCUSSION

### *The origin and behaviour of polyploid cherries*

*Tetraploids.* The origin and constitution of tetraploids can be established by a combined study from different angles. Representing each basic set of chromosomes by a capital letter, we have the following three types of tetraploids: **AAAA**, **AAAB**, **AABB**, which we expect to differ from one another in behaviour. Morphological studies, genetical and breeding experiments will help us to discover to which of these types a particular tetraploid belongs. But more precise information on the chromosome constitution and consequently on the origin of tetraploids can be obtained from the behaviour at meiosis.

It is now possible with certain reservations to interpret the cytological behaviour of tetraploids in terms of their chromosome constitution. Autotetraploids may differ markedly from one another in the proportion of quadrivalents formed. In the autotetraploid tulip *Tulipa chrysantha* for instance no quadrivalents are formed (Upcott, 1939), while in the autotetraploid *Solanum lycopersicum* (Upcott, 1935), *Primula sinensis* (Darlington, 1931a) *Datura Stramonium* (Belling, 1927) from one to twelve quadrivalents are formed. This variation in behaviour is due to factors which govern chromosome pairing such as chiasma frequency and distribution, position of the centromere, and whether pairing begins distally or proximally.

These considerations apply in the first instance as soon as the autotetraploid is produced. If the plant has passed through a number of sexual generations, other considerations must be taken into account. It is well known that the fertility of autotetraploids of recent origin is reduced as compared with the diploid prototype, owing to competition in chromosome pairing and abnormal disjunction. Sexual reproduction, however, allows selection to operate in the direction of increased fertility, lower chiasma frequency and lower quadrivalent formation. In other words, autotetraploids of remote origin will tend to behave more as diploids or as bivalent-forming allotetraploids, owing to genotypic selection.

Judging from the behaviour at meiosis, it is sometimes difficult to classify certain intermediate types as auto- or allopolyploids. For example an allotetraploid may show quadrivalents (*Crepis rubra*  $\times$  *C. foetida*, Poole, 1931; *Primula kewensis*, Newton & Pellew, 1929; *Aquilegia*, Skalinska, 1935) when the differentiation between the two sets of chromosomes is slight, while autotetraploids may show complete association as bivalents (*Tulipa*) if the chiasma frequency is low. Accurate classification in such instances, therefore, depends on whether it is possible to discover which of the two factors, chiasma frequency or chromosome differentiation, is the limiting factor. In certain cases, as we shall see later, it is possible to discover which of these two factors operates by studying the cytological behaviour among hybrids.

The Duke cherries, as we have seen, behave cytologically as autotetraploids. They show an average of six quadrivalents per nucleus, with a range from two to eight, and their chiasma frequency is high (1.59). They would seem to be of comparatively recent origin (Leroy, 1877; Mas, 1882; Hedrick, 1915) and as they are always propagated vegetatively, no opportunity has offered of selection for fertility by lowering of the chiasma frequency.

The sour cherries from their own cytological behaviour can readily be classified as autopolyploids, but they fall into the intermediate class referred to above. They form up to four quadrivalents with an average of 2.5, and their chiasma frequency is low, 1.32. They must therefore be considered as autopolyploids (probably of more remote origin than the Dukes) in which selection for fertility by lowering of the chromosome number has operated. This conclusion is substantiated when we consider the behaviour of their hybrids with the autotetraploid Dukes. These hybrids, as we have seen, do not behave in an intermediate manner, but like the Duke cherries themselves (two to seven quadrivalents,

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average 5.2). Evidently, although the chromosomes of the sour cherries fail to form many quadrivalents in the species, they are able to do so in the hybrid where the genotype is different.

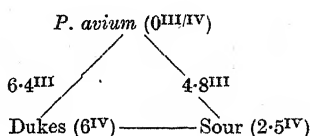
Low multivalent association in sour cherries therefore is due not so much to chromosome differentiation but rather to the genotypic constitution they have developed as the result of selection for fertility.

The behaviour of the hybrids between *P. avium* and the sour and Duke cherries gives additional confirmatory evidence of the constitution of these two tetraploid forms. The triploid hybrids (Table 8) evidently behave as autotriploids, although the proportion of trivalents is lower in the Sour-*avium* than in the Duke-*avium* crosses. Such difference in behaviour, however, is only slight.

Table 8

Triploids	Average III per P.M.C.	Variation
Dukes $\times$ <i>P. avium</i>	6.40	5-8
Sour $\times$ <i>P. avium</i>	4.84	3-7

The relation between the two tetraploid forms and the diploid *P. avium* can be illustrated in terms of multivalents by the following diagram:



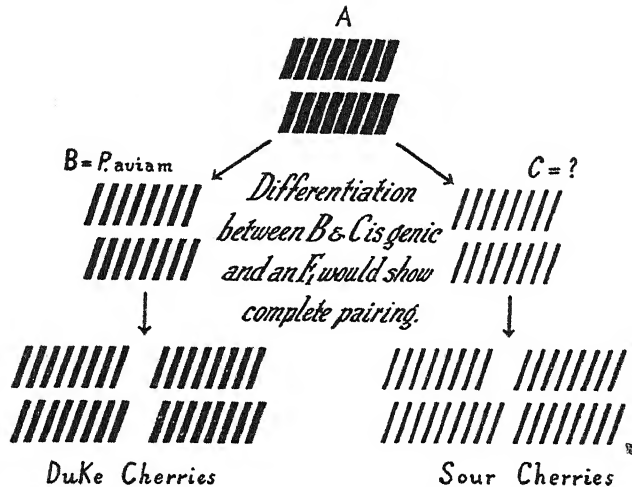
which shows also that the number of multivalents present in the triploids is higher than in the respective tetraploids owing to the absence of effective competition (Upcott, 1935).

From meiosis in the Duke-*avium* hybrid we must conclude that the Duke cherries are autotetraploid forms of *P. avium*. *P. avium* might on cytological grounds also be considered the diploid prototype of *P. cerasus*. This latter conclusion, however, is not compatible with the morphological evidence, the sour cherries differing in certain characters from both the sweet and Dukes. Two possible explanations can be suggested. First, that a change of morphology has occurred in *P. cerasus* through gene mutation. Secondly, and this is the more probable explanation, that *P. cerasus* is an autotetraploid form of a diploid cherry closely related to *P. avium*. This diploid prototype could differ only in a few genes, largely affecting morphology, hence chromosome differentiation would be very slight.

The probable origin and constitution of the three cherries: *P. avium*, Sour and Duke may be represented diagrammatically as in Text-fig. 10. It is possible that the sour cherries have arisen directly from the diploid form A without the intervention of the type C.

As regards the tetraploid species, *P. fruticososa*, we can now say that its similarity in behaviour to the sour cherries is a good ground for assuming that it is also autotetraploid; but since its behaviour with an allied species is unknown, we cannot test this possibility.

The tetraploid cherry *P. cantabrigiensis*, as we have seen earlier, behaves differently from the Sour and Duke cherries. The chiasma



Text-fig. 10. Origin and constitution of the Sweet, Duke and Sour cherries.

frequency is relatively high (1.43) but quadrivalents are formed only occasionally. These observations suggest an allopolyploid constitution for this species. The high chiasma frequency offers the opportunity for quadrivalent formation provided the four sets are homologous (AAAA). If on the other hand the tetraploid is of the constitution AABB the amount of quadrivalents will depend on the degree of differentiation between A and B and not on the chiasma frequency. The differentiation, however, between the two sets is not complete. The occasional formation of quadrivalents shows that autosynopsis as well as allosynopsis may take place between the four sets, but as happens with the polyploid *Erophila* (Winge, 1933) and allotetraploid *Aquilegia* (Skalinska, 1935) allosynopsis affects only a small portion of the chromosomes.

The triploid hybrid *P. cantabrigiensis* × *P. avium* shows, as we have

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seen, very poor chromosome pairing when compared with the triploids *Duke-avium* and *Sour-avium*. Only two trivalents are formed, and plates with no multivalents have been observed. Eight paired configurations are almost constantly formed, however, which indicates close homology between two of the three sets with the third set left wholly or partially unpaired. This recalls the behaviour of *Nicotiana tabacum* (Clausen, 1928) and *Crepis* hybrids (Hollingshead, 1930).

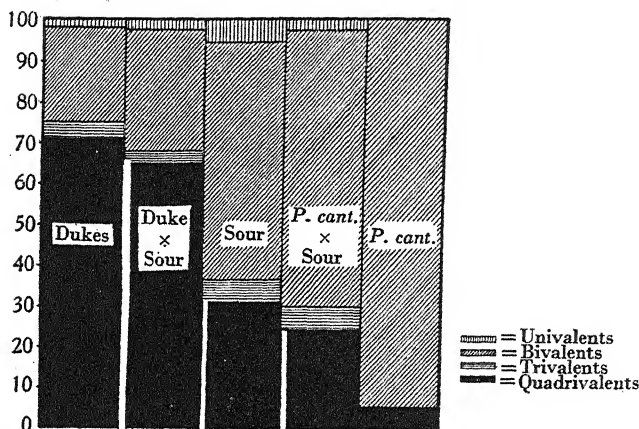
Obviously the bivalent pairing in this triploid may be due to auto-syndesis within the *P. cantabrigiensis* chromosomes rather than to complete affinity between the *P. avium* chromosomes and one of the *cantabrigiensis* sets. The hybrid *P. cantabrigiensis* × *Sour*, however, does not provide clear evidence for the allotetraploidy of *P. cantabrigiensis*, since the percentage of quadrivalents is almost as high as in the sour cherries (up to 4; average 2). For this reason an attempt was made to confirm its allopolyploid nature by studying the nucleolar relationships; as Bhatia (1938) for example concludes the allotetraploidy of *Triticum dicoccum* from a study of the number and sizes of nucleoli.

In *P. cantabrigiensis* we find as a rule one large nucleolus to which three pairs of chromosomes are attached, and since the original nucleoli have by this time fused into one it is probable that some bivalents have become detached. At first telophase four nucleoli develop in each daughter nucleus, two of which are larger than the others. This was the maximum number of nucleoli observed at this stage, and the size difference indicates an allotetraploid constitution for *P. cantabrigiensis*.

The analysis is complicated however by the occasional presence of as many as six nucleoli at prophase. It is possible that not all of them are real nucleoli, as the heterochromatin globules commonly seen at this stage may sometimes attain considerable size and appear as small nucleoli.

The cytological behaviour of *P. cantabrigiensis* supports the assumption that it is an allotetraploid of the type **AABB**, but the results obtained are insufficient to give conclusive evidence whether *P. avium* is one of its components or whether both sets making up *P. cantabrigiensis* belong to species differing from it. Anyhow the presence of up to four multivalents in both the tetraploid and triploid hybrids of *P. cantabrigiensis* with the sour and sweet cherries indicates that pairing may take place between certain of their chromosomes.

The pairing relationships and the constitution of all five tetraploid species and hybrids discussed so far is given in the diagram below, which may serve as a general conclusion.



Text-fig. 11. Pairing relationships of five tetraploid cherries.

*Fertility in relation to cytological behaviour*

*Triploids.* All triploids are more or less sterile compared with the corresponding diploids. The most important factor in determining the degree of sterility is the segregation of the extra set of chromosomes. For example, triploid cherries with random distribution of the odd eight chromosomes will give 8- to 16-chromosome gametes, varying in viability, and only the more favoured genotypes will be expected to survive. In *Pyrus* (Thomas, unpublished) all the different classes of gametes can function, though in unequal numbers. In cherries on the other hand selection is so effective that all the aneuploid classes are inviable. Random distribution of the eight chromosomes would give two balanced gametes in a population of 256, or 0.8 %. The actual percentage of pollen germination is 1 %, which is close to the expectation. Further, two seedlings from a tetraploid  $\times$  triploid cross were found from somatic metaphases to be tetraploids (family 2/39) which shows that only 16-chromosome gametes from the triploid parent had functioned. The absence of 8-chromosome gametes is apparently due to the effective competition of the diploid over the haploid gametes in the tetraploid style (Raptopoulos, 1941).

As a result of this strict elimination of aneuploid gametes, no aneuploid cherries are known in nature or in experiments, apart from one 25-chromosome plant reported by Okabe (1928).

*Tetraploids.* The tetraploids have comparatively few univalents



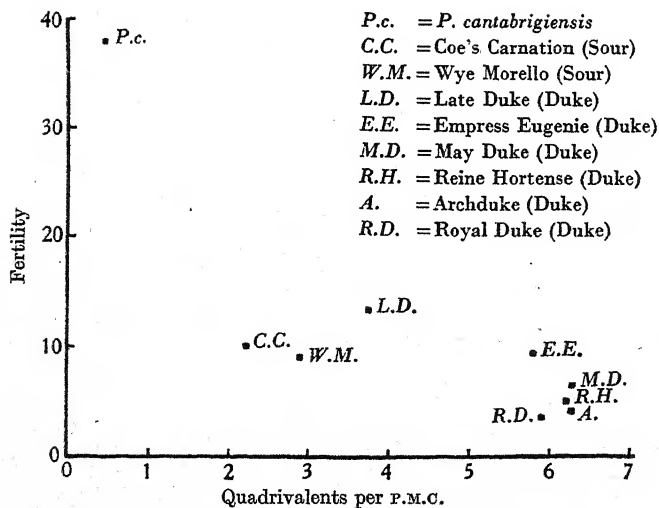
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(2.7 %), but since quadrivalents are present in a high degree, aneuploid gametes can be produced through irregular disjunction.

Table 9 gives the data for the tetraploid species and varieties for which fertility numbers are available, and Text-fig. 12 illustrates the

Table 9

Species or variety	No. of quadrivalents per P.M.C.	Fertility %
<i>P. cantabrigiensis</i>	0.44	38.0
Sour:		
Coe's Carnation	2.20	10.0
Wye Morello	2.88	9.0
Dukes:		
Late Duke	3.75	13.5
Empress Eugenie	5.80	9.6
Royal Duke	5.90	3.6
Reine Hortense	6.20	5.1
May Duke	6.25	6.8
Archduke	6.25	4.3



Text-fig. 12. Relationship between fertility and proportion of quadrivalents in nine tetraploid cherries.

relationships between fertility and proportion of quadrivalents graphically. The fertility data were obtained from the breeding experiments carried out here over a period of 25 years. These data have shown that the fertility, expressed as a percentage of fruits set and matured of the flowers pollinated, decreases from the sweet cherries to the Duke cherries with the sour cherries as intermediates. *P. cantabrigiensis*, recently

introduced into the experiments, proved to be as highly fertile as the most prolific of the sweet cherries. A certain amount of variability was found in the fertility percentages from year to year and among different cross-pollinations, apparently due to environmental conditions and incompatibility factors.

Text-fig. 12 clearly shows a general reduction in fertility with increase in the proportion of quadrivalents. The difference between the fertility of *P. cantabrigiensis* and the sour cherries is very large, whereas the drop from the sour cherries to the Dukes is not so striking. In *P. cantabrigiensis*, as we have seen, quadrivalents are only occasionally formed, so that most of the pollen mother cells must show a 16-16 disjunction. In sour cherries on the other hand the average of quadrivalents is 2.5 per cell and no cases are recorded where the association is 16<sup>II</sup>.

A discrepancy found between the fertility numbers of certain varieties having the same average number of quadrivalents is possibly due to the manner of disjunction of these latter. It was found, for instance, that 71 % of the quadrivalents of Late Duke and 74 % of Empress Eugenie have an orientation on the spindle which may result in an equal 2 × 2 disjunction of their chromosomes. This proportion falls to 63 and 69 % in Wye Morello and Royal Duke respectively.

The conclusion to be drawn from these results therefore is that the important factor in causing a drop in fertility is the constant presence of quadrivalents in the pollen mother cell, and that increase in the number of quadrivalents does not cause a proportional decrease in fertility. Certain discrepancies in the correlation between number of quadrivalents and fertility among certain plants may be due to differences in the relative co-orientation of their multivalents.

#### SUMMARY AND CONCLUSIONS

1. An account is given of the meiotic behaviour of diploid, triploid and tetraploid species and hybrids of cherries, with a special consideration of the numerical data obtained.

2. From these accounts the conclusion is drawn that the Duke cherries are autotetraploids with a high chiasma frequency (1.59) and a high percentage of quadrivalents (average 6).

3. The sour cherries are also considered to be autotetraploids, but they have a low chiasma frequency (1.34) and also a relatively low proportion of quadrivalents (average 2.5).

4. This conclusion is supported by the behaviour of the Duke × Sour hybrids. Here the chromosomes of the sour cherry under the hybrid

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conditions are able to produce as many multivalent configurations as the Dukes themselves.

5. It is therefore considered that the low quadrivalent formation in sour cherries is due to selection for low chiasma frequency rather than to differentiation between the chromosomes.

6. The triploid hybrids *Duke-avium* and *Sour-avium* support the above conclusions. They behave as autotriploids with an average of 6.4 and 4.8 trivalents respectively.

7. The cytological evidence shows that the Duke cherry is an autotetraploid form of *P. avium*, which conclusion agrees with its genetical similarity to *P. avium*.

8. The cytological evidence indicates that the Sour cherries can also be considered as autotetraploids arising, not from *P. avium* itself, but from a postulated species separated from *P. avium* by certain differences of morphology, and having a common ancestry with it.

9. *P. cantabrigiensis* from its high fertility and general cytological behaviour is considered to be an allotetraploid.

10. No aneuploid gametes of cherries are viable. This conclusion is drawn from the present investigation and from breeding experiments with *Prunus* species.

11. An inverse relation was found between the number of quadrivalents and the fertility in nine species and varieties of tetraploid cherry.

I am indebted to the British Council for enabling me to carry out this work at the John Innes Horticultural Institution.

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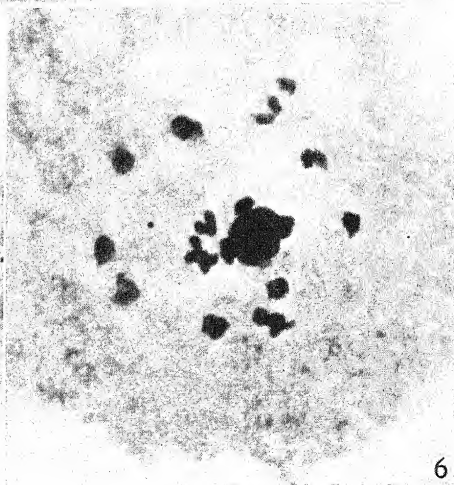
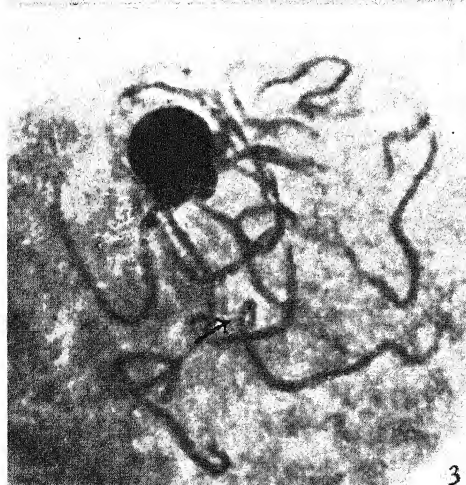
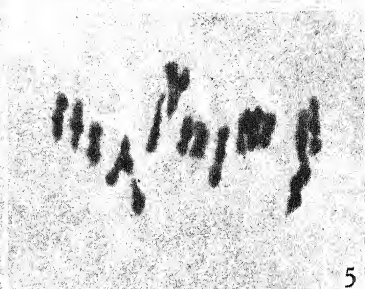
### EXPLANATION OF PLATES 5 AND 6

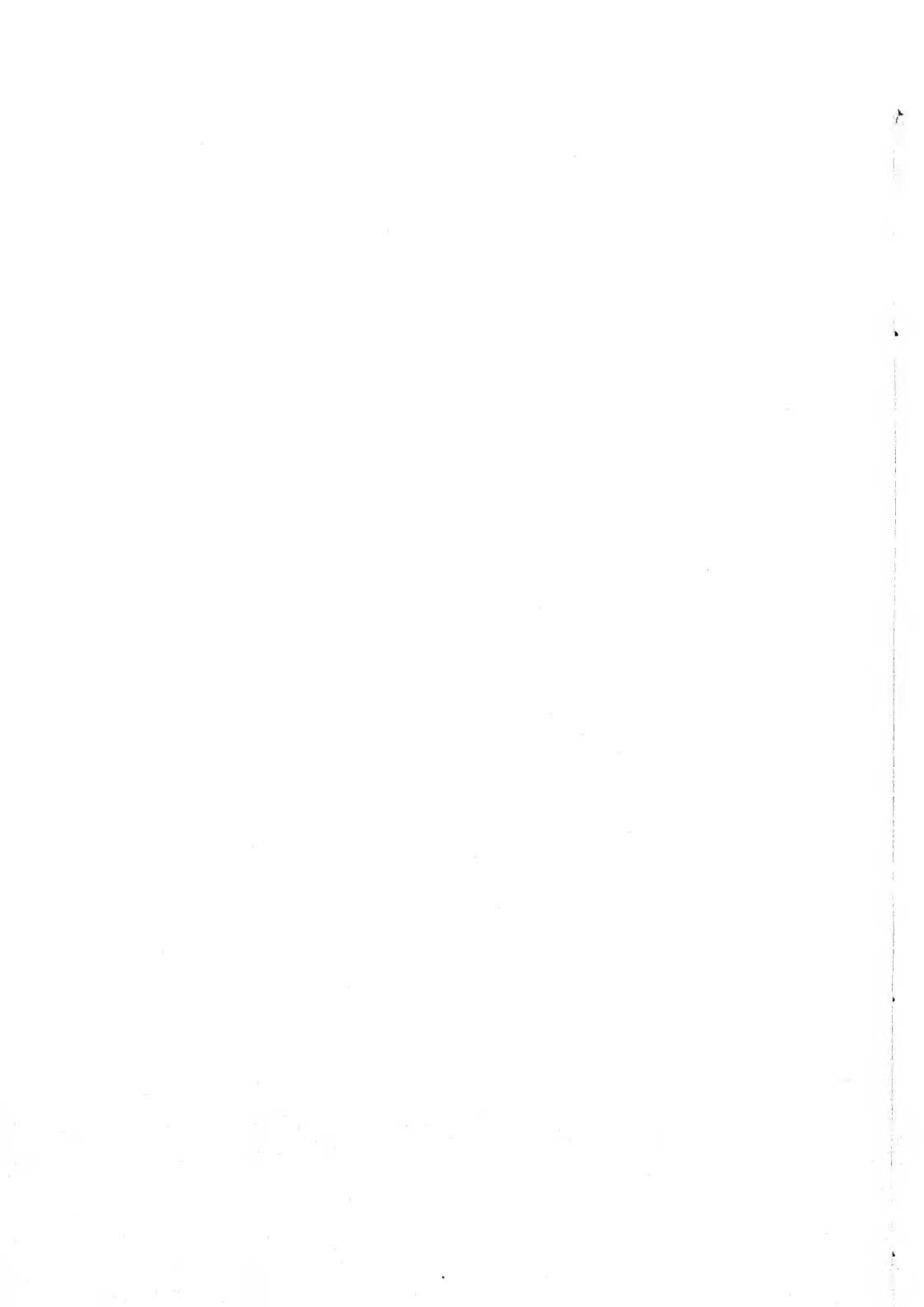
#### PLATE 5

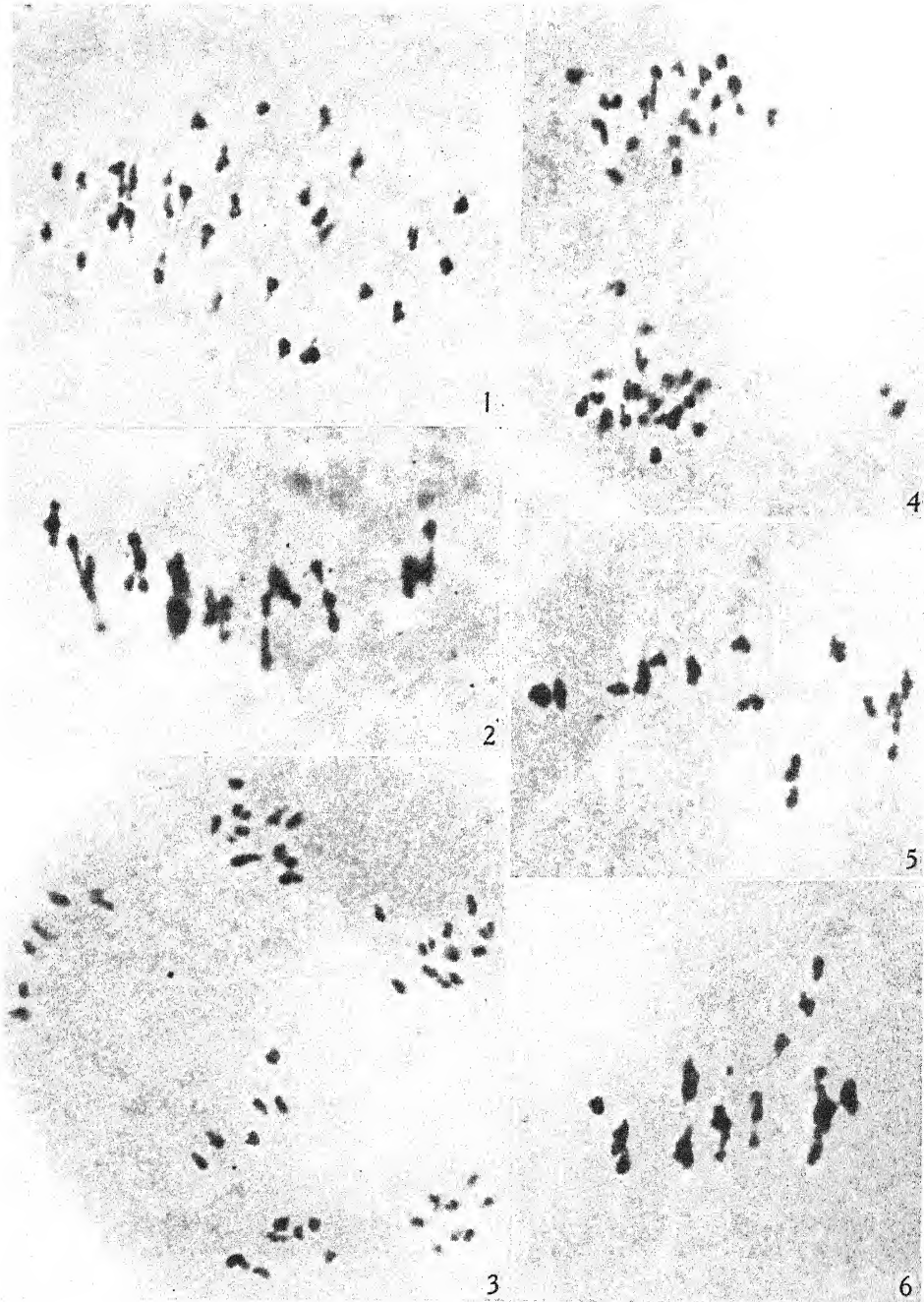
- Fig. 1. First metaphase of the Duke cherry Reine Hortense, with eight quadrivalents.  
Fig. 2. First metaphase of Wye Morello (Sour). Three quadrivalents, one trivalent, eight bivalents and one univalent.  
Fig. 3. Pachytene of *P. cantabrigiensis*. Unpaired segment.  
Fig. 4. Late anaphase in the sour cherry, Coe's Carnation. Differential disjunction, lagging univalents.  
Fig. 5. First metaphase of the tetraploid hybrid *P. cantabrigiensis* × Sour cherry. Three quadrivalents, ten bivalents.  
Fig. 6. Diakinesis in *P. cantabrigiensis*. Three bivalents attached to the nucleolus.

#### PLATE 6

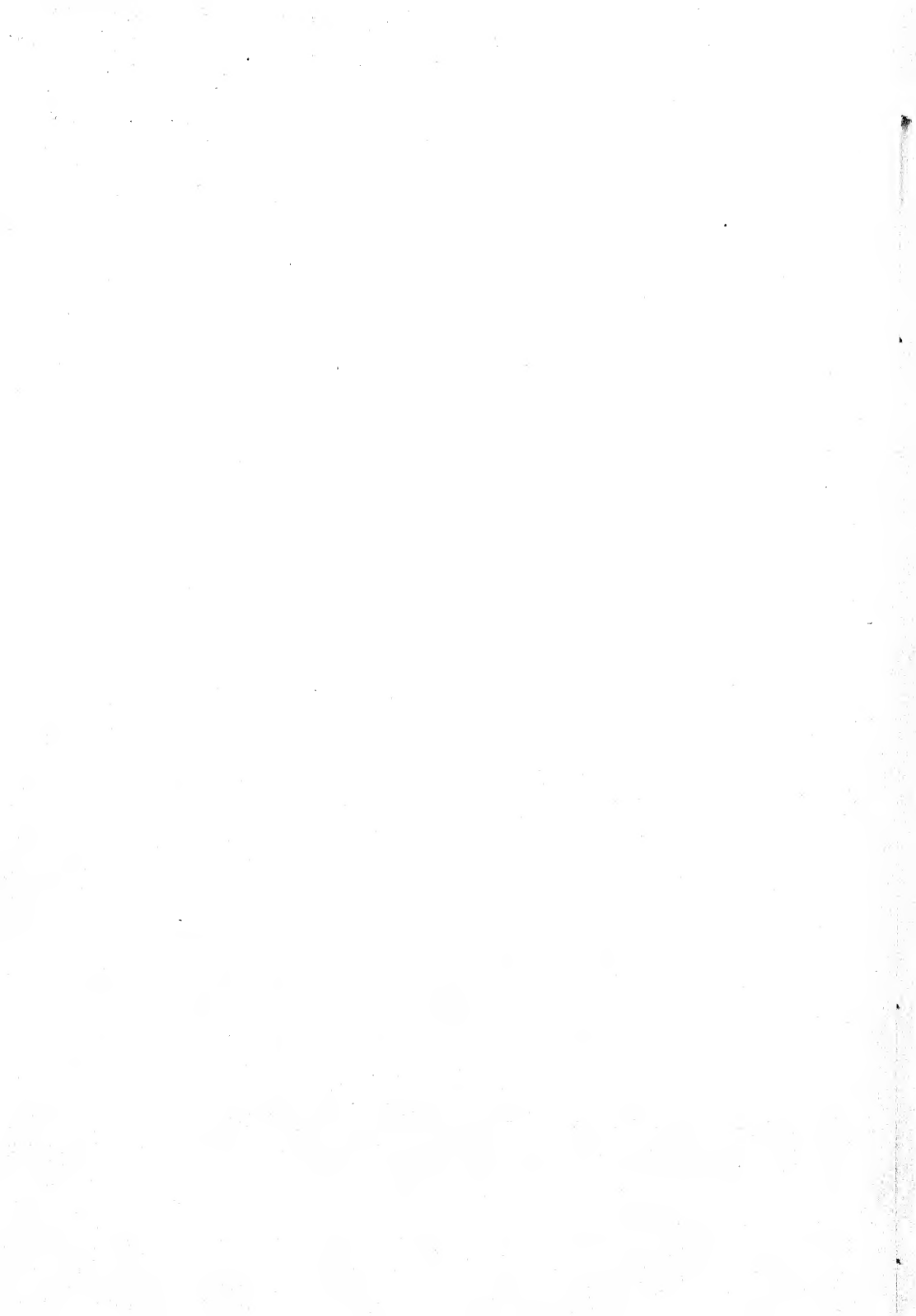
- Fig. 1. Anaphase of Coe's Carnation (sour). A quadrivalent not yet disjoined.  
Fig. 2. First metaphase in the tetraploid hybrid Duke × Sour. Seven quadrivalents, two bivalents.  
Fig. 3. Six groups of second telophase chromosomes in the triploid hybrid *P. cantabrigiensis* × *P. avium*.  
Fig. 4. Late second anaphase of an incomplete restitution nucleus of the triploid *P. cantabrigiensis* × *P. avium*.  
Fig. 5. First metaphase of the triploid *P. cantabrigiensis* × *P. avium* (9/34). Three trivalents, five bivalents, five univalents.  
Fig. 6. First metaphase of the triploid Duke × *P. avium*. Seven trivalents, one bivalent, one univalent.











# OPERATIONS ON THE PUPAL WING OF *DROSOPHILA MELANOGASTER*

By A. D. LEES

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(With Plate 7 and Sixteen Text-figures)

## 1. INTRODUCTION

FEW animals have proved to be favourable enough material to yield information in the fields of both genetics and embryology. Certainly, when this is possible, it is seldom combined with any knowledge of the relationship between gene processes in development and the interrelations of organs and tissues in normal development. That is to say, the field of experimental embryology is usually left unexplored by virtue of the great technical difficulties encountered. This account is an attempt in some measure to fill this gap. The technique used, although necessarily the simplest employed by experimental embryologists, has given results which are consistent in themselves, and are of value in interpreting both the processes of embryology and phenogenetics.

The wing of *Drosophila melanogaster* is an example of an animal organ which is influenced by a large number of genes whose genetic effects are already well known. Moreover, recently Waddington has published a very complete account of both the normal development of the pupal wing of *Drosophila*, and the development of some twenty-eight mutant characters affecting the wing (Waddington, 1940). It is on this account that the present study is based, for without such a foundation it would have had little value.

## 2. TECHNIQUE, AND CLASSIFICATION OF RESULTS

All operations were made on a long inbred stock of Oregon R, and the pupae were timed to the nearest hour in an incubator running at  $25 \pm 0.3^\circ \text{C}$ . No discrepancies in the developmental times were noted. In all cases the operations were made with a very finely pointed steel-needle; such wounds healed rapidly without being covered by adhesive substances. At first most of the pupae were fixed at about 40 hr. when the wing is flat, and most of the definitive wing characters have been differentiated. Later it was found that, if kept in moist tubes, about

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90 % of the wounded pupae would hatch. From then all the pupae were allowed to hatch, and the wings were mounted in Canada balsam or de Faure's medium. Examination of the imaginal wings offered greater possibilities of interpreting detailed structure such as hair distribution, etc. All text-figures were drawn at bench level with an Abbe camera lucida. Unless otherwise stated the magnification is  $25\times$ .

In all, over one thousand successful operations were made, most of these at the 0-3 hr. stage, as this time group gave a very high percentage of normal wings. After eversion through the peripodial membrane at about 4 hr., the wing becomes rather transparent, and it is difficult to wound the wing at every operation: however, there is reason to believe that every wound from this stage onwards produces a defect in the adult wing. After about 14 hr., when the wing membrane has secreted a chitinous sheath, operations can be, to some extent, localized. Only a few operations were made after 25 hr., as the only effects were small deficiencies in vein or membrane showing no sign of either repair or regulation.

The results are for convenience grouped chronologically into three main sections. This is not, however, an artificial classification, as most of the effects produced are confined to the time group in which they occur, and may be correlated with the embryological history of the wing. Group A (0-3½ hr.) corresponds to the invagination of the wing bud, its eversion through the peripodial membrane, and the close apposition of the dorsal and ventral epithelia, with the accompanying clarification in the positions of the prepupal veins. Group B (3½-6 hr.) fall within the period when the wing is becoming flatter, and of greater area through cell multiplication and cell rearrangement. Finally, group C (6-21 hr.) corresponds to the time when the wing is becoming inflated, the two surfaces being forced apart. This process reaches its zenith at about 19 hr., when all traces of the prepupal venation have been obliterated. At 21 hr. the two epithelia are once more beginning to be brought together with the consequent appearance of the definitive imaginal vein pattern.

### 3. EXPERIMENTAL RESULTS

#### A. *Operations on 0-3½ hr. pupae*

At this stage the imaginal wing buds are lying just under the anterior dorsal surface of the prepupa. They are very conspicuous, and the needle can be seen to pass through the buds at every operation. However, out of a total of 659 operated pupae, only eighty-three proved to have wing

defects in the adult. Of these 465 were operated on at 0-1 hr. and produced eighty injuries, while 2-3½ hr. operations gave only three out of 194. This peculiarity may at least be partly explained by the fact that by about 3 hr. the wing has probably ceased to invaginate further, and also that the wing surfaces have come together. This will, however, be further considered under the separate effects produced by the operations. These are, in the main, five in number: (a) scalloping effects, (b) disruption of the venation, (c) *fused* effects, (d) *tilt* effects, and (e) vesiculations.

Throughout this set of operations the imaginal wing membrane inside the margin is found to be entire. From 0 to 3½ hr., therefore, the epithelium must possess a considerable capacity for regeneration. Certain irregularities in this process are, however, noticeable; for instance, in some wings the site of the wound is marked by hairs which are grouped in pairs or sometimes in threes. Probably they have arisen from a single cell. Moreover, there is a clear-cut difference in potentiality between the dorsal and ventral surfaces, for the latter never exhibits any duplication of hairs. Also there is often a size difference between the hairs covering the wound and those on the rest of the wing surface, and this seems to vary independently on the two surfaces. For instance, the hairs on the ventral side of the wound may be small while those on the dorsal side are normal; or conversely, in another example the dorsal hairs are represented by pits while the ventral hairs are normal.

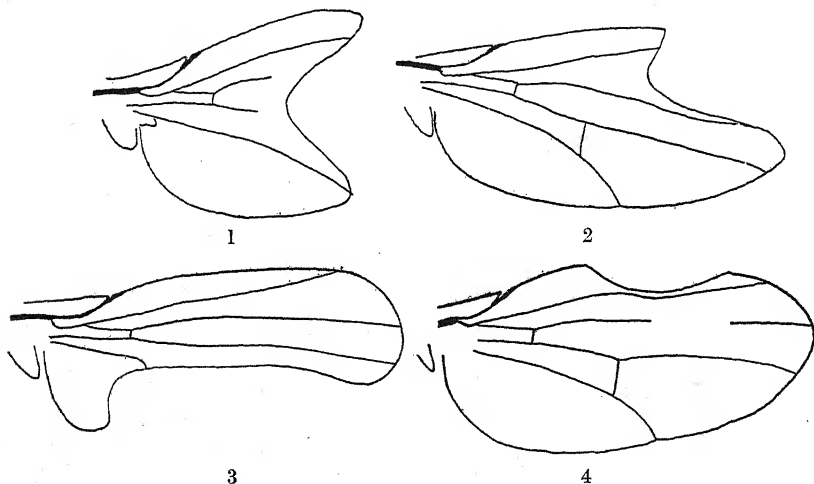
There are also considerable differences in the cell size and hair orientation of the two surfaces. This is an effect occurring almost exclusively in those wings which exhibit vein irregularities, and can be more usefully considered in subsection (b).

(a) *Scalloping effects* (Text-figs. 1-4; Pl. 7, figs. 1, 2).

In these operations a portion of the wing margin is removed, leaving the centre of the wing more or less intact. In appearance they may simulate such mutants as *nicked*, *notched*, *Xasta*, etc. There seems to be very little regulation over the surface of the wound, and there is no regeneration of the lost margin. It can be said, therefore, with some degree of certainty, that at the stage of the 0 hr. prepupa the wing margin is determined. This does not apply to the general membrane within the margin, since it is able to regenerate until a much later date, provided it is not prevented by mechanical factors (such as tissue tensions, etc.). There is, however, no visible sign of any difference between the marginal and interior epithelial cells until the hairs are formed at about 45 hr. when the former are seen to bear much longer hairs than those of the latter.

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The small number of scalloped wings arising from these operations makes it seem probable that they do not arise in the obvious way of having a piece knocked off their edges by the needle. Considering 0 hr. operations, out of a total of 465 wings operated on, which resulted in eighty injuries, only twenty-four (or 5 %) were scalloped. From 2 to 3 hr. operations only two out of 194 (or 1 %) were so injured. This indicates that the process leading to the scalloping effect was declining from 0 to 3 hr. Now it has been pointed out by Waddington that the wings of the scalloped mutants are not the result of any degeneration of the margin as postulated by Goldschmidt (1935, 1937). Waddington has demonstrated that they are caused by an alternation in the shape of the area



Text-figs. 1-4. Scalloped wings resulting from 0 hr. operations. Text-fig. 4 shows the co-existence of scalloping of the anterior margin and a tilt effect of  $L_3$ .

of invagination of the wing fold in relation to the invaginating material. Thus to produce a long, thin *Beadex*<sup>J</sup> wing with anterior and posterior margins missing, the mouth of the invaginating pocket is narrowed. From this it follows that the veins are in some way determined on the invaginating epithelium, since there is no adaptation of the veins to the new size of the wing. The ends of the veins are simply removed together with the margin.

It seems probable that in these operations the scalloping is effected by altering the position of the invagination lip. This is strongly borne out by the fact that this effect almost ceases to occur at 3 hr. when the wing fold has ceased to invaginate further, and when an alteration in the lip would no longer be expected to produce any effect on the adult wing. At a later

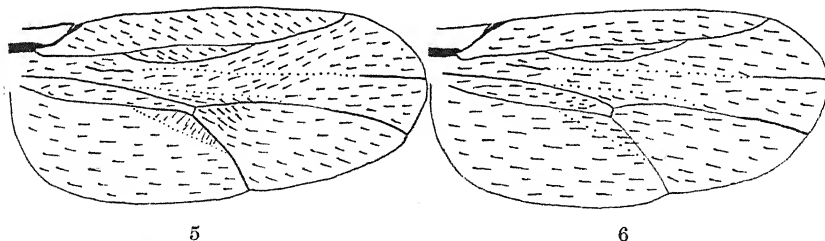
stage in the pupal history, from 14 hr., when the wing lies in a chitinous sheath directly under the puparial wall, the location of the operation can be selected with some exactitude. Operations on the margin then produce scalloped wings of a similar appearance. However, they are the result of a totally different process, namely, direct injury to the margin, and they are easily reproducible.

The hypothesis advanced here is supported by the fact that scalloping and tilt effects may occur in the same wing although only one operation is made (Text-fig. 4). It will be shown that a tilt wing with a central portion of a vein missing is probably produced by the needle passing directly through, or close to, the vein material. Consequently it is difficult to imagine how a single prick could injure two parts of the wing lying in the same plane, and leave the intervening area intact, unless the scalloping was effected by an indirect process such as that postulated above.

Such scalloped wings frequently show certain secondary effects such as localized contractions of the epithelium. These phenomena are not peculiar to this time group, and are more conveniently considered in section C.

(b) *Disruption of the venation* (Text-figs. 5-7; Pl. 7, figs. 3-7).

This effect, frequently accompanied by scalloping, occurred in ten out of 659 operations. These all resulted from 0 to 1 hr. operations; the 194 2-3 hr. wings included no such defects. The appearance was generally that of a disturbance in the direction of the longitudinal veins  $L_3$ ,  $L_4$ ,



Text-figs. 5, 6. Vein disruption resulting from a 0 hr. operation. Text-fig. 5 represents the dorsal, and Text-fig. 6 the ventral surface. The veins are drawn similarly in both figures. The partially induced veins on the ventral surface are indicated by dotted lines. For explanation of the hair distribution and orientation see text. This wing is also figured in Pl. 7, fig. 3. ( $\times 35$ .)

and  $L_5$ . In many cases these may be broken at one or more points; the ends of the free section of vein join up with a neighbouring vein. For instance, the central portion of  $L_3$  may, at each end, be confluent with  $L_2$  (Text-figs. 5, 6; Pl. 7, fig. 3), or  $L_3$  may be dragged posteriorly to join the

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tip of  $L_4$ , and from there to the margin form a single vein (Pl. 7, fig. 4). In most of these examples where the vein material is abnormal in position, the normal track of the vein is indicated by a faint line or ridge bearing a row of orientated hairs. This is particularly clearly shown in cases where a vein may apparently bifurcate and then rejoin (Pl. 7, fig. 4). It is difficult to make out by focusing on which surface the faint "vein" is located, but it seems probable that it is the ventral. The thick part of the vein may be readily indicated in  $L_3$  by the presence of the chordotonal organs; it is as broad and well-chitinized as the normal vein.

Now it has been pointed out by Waddington that the lack of vein regulation in the scalloped mutants makes it necessary to assume that the veins are in some degree determined at the time of the invagination of the wing. However, if the veins were determined on both surfaces separately, it is inevitable that, in some scalloped mutants, material which would normally form part of the top surface would be brought on to the bottom surface and cause a complete derangement of the pattern. This, however, never occurs. It was suggested therefore that the veins were determined on one surface, and that, on coming in contact with the other member, they were able to induce the formation of veins on the opposite surface. In the 0 hr. prepupa there is visible evidence that the dorsal epithelium has a peculiarity of structure in the presence of two ridges lying above the future  $L_3$  and  $L_4$ . At this stage no prepupal veins can be made out in whole mounts or sections, but the ridges may denote an embryological determination of these veins.

This hypothesis appears probable on the basis of the present results. It is possible to conceive that an operation would in a certain number of cases shift the two surfaces relatively to one another. In such examples the rather faint ridge of the half-vein on the ventral surface would represent the relict of an induction process only half-completed. The main vein on the dorsal surface has influenced new epithelial cells on the ventral side, and appears as thick as a normal vein. It is noteworthy that at 3 hr., when the vein surfaces have come securely together, no such defects are ever found. Also these induction defects are strikingly absent with regard to  $L_2$ . Now  $L_2$  has no prepupal representative, and is produced in an entirely different way by the fusion of small lacunae between the bases of the epithelial cells. Further, the exact course of  $L_2$  may be considerably modified at a much later date as shown by 5 hr. and later operations. From these facts it can be seen that an early shifting of the two surfaces would scarcely be likely to have any effect on  $L_2$ . The hypothesis in general appears extremely probable in one case (Pl. 7,

fig. 5) where the distal edge of the wing has been removed, and where the two wing surfaces have obviously not become properly accommodated. This is reflected in the rumpled appearance of the wing membrane and in the bifurcation of the tips of the veins  $L_3$ ,  $L_4$  and  $L_5$ .

Further support is forthcoming from a study of the hair distribution on the two wing surfaces; if these have been moved sideways relatively to one another, one would expect to find some evidence of stretching and hair disorientation. Such conditions are usually found in this class of wings, and may provide a clue as to the position and extent of the lateral movement.

As an example the structure of one such wing may be analysed (Text-figs. 5, 6; Pl. 7, fig. 3). In the diagrams no attempt has been made to draw all the hairs, and it is only desired to convey an impression of their spacing and orientation. It can be seen that there is considerable vein irregularity; the centre of  $L_3$  is attached at both ends to  $L_2$ ,  $L_4$  has a large central gap, while  $L_5$  at the region of the posterior cross-vein has apparently been shifted anteriorly. As in the other examples of this type of injury, the movement of  $L_5$  has left a line of rather weak vein material on the ventral surface in its original position.

In the normal wing all the hairs point towards the wing tip and lie approximately parallel to the long axes of the cells. Presumably there is a causal relationship between the two. In the triangular area posterior to  $L_5$  the hairs are arranged at right angles to their normal orientation, indicating the reality of the forward shift of the dorsal surface. This change in direction of the hairs is only continued to the line of the half-induced  $L_5$  on the ventral surface; evidently the surfaces were adherent along this line. A similar stretching of the cells is indicated anterior to the broken section of  $L_3$ , where the hairs are curved round so as almost to run in a transverse direction. There is also a marked increase in hair density anterior to the posterior cross-vein as if the forward movement of  $L_5$  had been partially retarded.

On the ventral surface, however, there is very little change in either the hair orientation or spacing, and this is correlated with the normal position of the induced section of  $L_5$ . Evidently the series of events can be summarized thus: the dorsal surface had begun to induce the veins on the ventral epithelium lying in contact with it, when the passage of the needle separated them anteriorly to the half-induced  $L_5$ ; local differences of tension in the dorsal surface caused irregularities of cell spacing and orientation as the tissue was moved bodily forward. This forward movement broke  $L_3$  centrally, and the free ends joined  $L_2$ . The ventral surface



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was relatively unchanged. Finally, the two epithelia were joined together again, and the process of induction was repeated on different cells in the ventral layer, the whole of  $L_5$  being reconstituted.

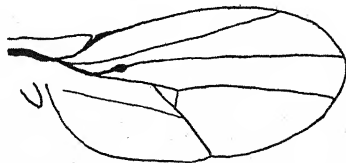
An alternative hypothesis might be put forward, namely, that the operation had in some way stirred up the determined vein material. This, however, is negated by the infrequency of the defect, and by the unlocalized nature of the vein disturbance. In several wings the extent of the wound can be seen, since irregularities of hair disposition and size occur. It is very small when compared with the wide area over which the veins may be affected. Further evidence against this hypothesis is provided by operations which pass directly through vein material (tilt effects), and which may produce no dislocation of the two surfaces, and no general disturbances of the venation.

Some of these wings bear considerable resemblance to the mutant *venae abnormeis* (Timoféeff-Ressovsky, 1927). This character is extremely variable; in some flies, pieces are missing from the wing veins, and in others there seems to be some extra venation. A very constant feature is, however, the branching and rejoining of the veins. In Timoféeff's illustrations one branch, as in these operations, is markedly fainter than the other. It is also noteworthy that in none of the ten wings figured is  $L_2$  affected.

In two cases operations have resulted in wings approaching the condition found in the mutant *venae plexoides*, where there are one or more small extra veins arising from the posterior cross-vein. This represents a tendency found in wild-type flies, and cannot be described as peculiar to the operated wing.

### (c) *Fused effects* (Text-fig. 7; Pl. 7, fig. 8).

In three examples from the 0 to  $3\frac{1}{2}$  hr. group of wings, the bases of  $L_3$  and  $L_4$  are confluent, resembling the condition in the mutant *fused*. In normal adult wings these veins are separate to their bases, although in the

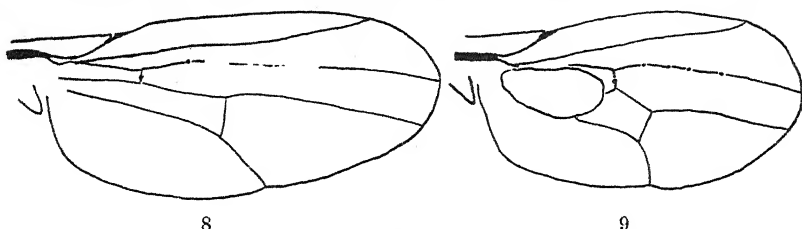


Text-fig. 7. Wing from a 0 hr. operation showing the *fused* effect where the bases of  $L_3$  and  $L_4$  are confluent. This is complicated by lateral shifting of the two epithelia which caused  $L_5$  also to become fused with  $L_3$  and  $L_4$ . The original base of  $L_5$  is indicated by a faint, partly induced vein on the ventral surface. There is also some weakening of  $L_3$ .

prepupal wing they arise from a common median prepupal vein. The explanation of *fused* and of these operated wings may lie in the retention of the prepupal dichotomy. On the other hand, a contributing factor may be the relative movement of the wing surfaces as described in section (b). This movement would be more important in the narrow base of the wing. Indeed, in one case  $L_5$  is also fused to  $L_3$  and  $L_4$ , and this vein has evidently been shifted laterally since its original bed is indicated by a faint ventral vein (Text-fig. 7).

(d) *Tilt effects* (Text-fig. 8; Pl. 7, figs. 9-10).

This condition is named after the mutant *tilt*, in which part of the middle region of  $L_3$  is missing. Among the operated wings, some interruptions of other veins are also included under this heading; I have found eight tilts of  $L_3$ , one of the distal end of  $L_4$  and three of the proximal



Text-fig. 8. A *tilt* effect resulting from a 0 hr. operation showing the deficiency of the vein  $L_3$ . Relevant chordotonal organs are represented by dots; they are absent distal to the injury.

Text-fig. 9. A 12 hr. operation resulting in a large hole breaking the base of  $L_3$ . Chordotonal organs are present distal to the breakage.

portion of  $L_5$ . These defects are remarkably constant in position; thus the  $L_3$  tilts are always found distal to the anterior cross-vein and occupy a section approximately in the centre of that vein. In most cases a faint ridge or double line of hairs marks the position of the missing portion of vein. Similar faint indications are found in mutants such as *veinlet* and *tilt*, and in these it is known that the vein is originally intact; the disappearance of the affected sections does not occur until about 20 hr., and is caused by a failure of the thickened epithelium above the veins to retain its characteristic histology. The cells in fact behave like cells of the intervein epithelia, and put out basal processes which join those protruded from the cells of the opposite surface and thus obliterate the lumen. It seems most probable that the developmental processes of the operated tilts are essentially the same.

The reason for this failure of persistence of the vein character is somewhat obscure. The tilts are most common in wings operated at

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0-3 hr., although not confined to them. Their relative frequency at this time may be correlated with the fact that the regenerative power of the epithelium is not counteracted by any great tension in the membrane. The actual production of the effect might either be supposed to be direct, by an injury of the cells which should build the missing part of the vein, or it might be imagined to occur in some more indirect manner. Against the hypothesis of a direct effect is the fact that during the early prepupal period, when the tilts are initiated, there is no distinction between the basal parts of  $L_3$  and  $L_4$ , which are united in the median prepupal vein; it is difficult therefore to understand how the injuries can be confined to  $L_3$ . But it is not at all certain how the proportions of the prepupal veins are represented in the pupal pattern of venation. Thus, if the injuries to  $L_3$  were made distal to the junction of  $L_3$  and  $L_4$ , one would not expect  $L_4$  to be affected. This would necessitate a greater degree of elongation of the wing distal to the junction to satisfy the spatial relations, for the bifurcation of  $L_3$  and  $L_4$  takes place approximately in the centre of the prepupal wing while the tilts may extend to within a third of the wing length from the base. The one tilt of  $L_4$  that has been recorded co-exists with a normal tilt of  $L_3$ , and might be taken as evidence of injury to the common base of  $L_3$  and  $L_4$ ; however, the deficiency of  $L_4$  is much shorter than that of  $L_3$ , and appears to represent a thinning of the vein rather than a true deletion such as occurs in the characteristic tilts. There is, nevertheless, positive evidence that some sort of indirect effect is involved, since in several cases the location of the prick can be determined by the presence of deranged or duplicated hairs, and although it is in the neighbourhood of the defect, it is less extensive than the region in which the vein is missing. One must conclude that an injury in the region of a vein may inhibit the differentiation of the vein material over a rather wide area. Certainly, direct local injury to a vein prevents its normal development for some distance each side of the actual wound.

It is extremely suggestive that the effect is most easily produced in  $L_3$  and seems to be altogether absent from  $L_2$ . The latter vein is developed in quite a different way from all the rest, and probably differs considerably from them in its determinative relations (p. 128).

The former,  $L_3$ , is remarkable in that it is the only one of the longitudinal veins which contains a nerve and tracheole, and bears chordotonal organs (with the possible exception of  $L_1$ ). We shall see later (p. 132) that there is considerable evidence that the chordotonal organs are dependent on something which grows down the vein, and this is presumably the nerve. It is easiest to interpret the frequency of tilt effects

in  $L_3$  by the supposition that the differentiation of the vein is also partly dependent on the nerve, and in the absence of the nerve is more readily inhibited than is the differentiation of  $L_4$  and  $L_5$ . Prickings which happen to hit the nerve would then have considerable effect on  $L_3$ , while similar operations in the neighbourhood of  $L_4$  and  $L_5$  may in the majority of cases leave them unaffected.

(e) *Vesiculation effects* (Pl. 7, fig. 11).

Under this heading are grouped a rather heterogeneous collection of wings in which the wing surfaces have not become completely co-adapted. In some cases the crumpled appearance is simply due to lack of expansion after emergence, but generally the effect is a fairly definite one, that of wings exhibiting large blister-like patches centrally. The appearance is very similar to the mutant *vesiculated* (Evang, 1925). There is no evidence that the blisters are caused by the trapping of lymph between the wing surfaces such as occurs in *bloated*, and the most likely explanation is that the effect is produced during contraction from the inflated stage when the injury acts as a focal point about which contraction fails to occur normally. The orientation of the hairs on the two surfaces is destroyed, and gives the impression that localized contractions in the two epithelia have caused the rumpled effect which is so characteristic of these vesiculations.

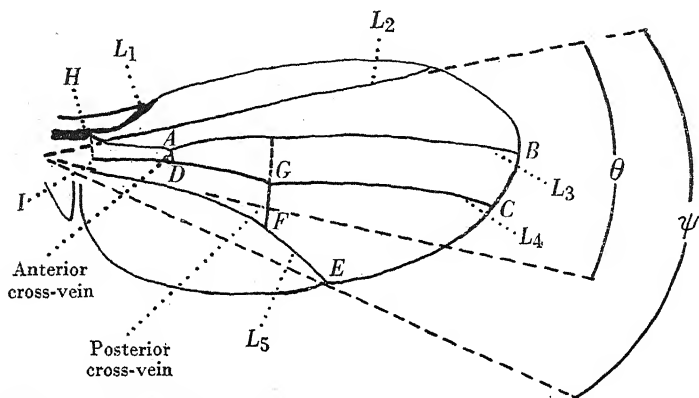
B. *Operations on 4-6 hr. pupae* (Text-figs. 11, 12; Pl. 7, figs. 12, 13)

Operations at this time produce wings of a very characteristic shape; they are very short, and are usually considerably broadened. In some extreme cases the wing may be nearly circular. The venation is in most cases normal in appearance although distorted in relation to the wing membrane. The exact site of the wound cannot usually be seen, as the epithelial cells have at this time a high capacity for regeneration; holes are very infrequently found. The general shape of the wings and the arrangement of the veins can be seen to be influenced by two main processes which will now be examined in more detail.

In the *dumpy* wing mutants of *D. melanogaster* the shape of the wing may be considerably altered although the wing margin is still entire. Waddington has shown that this is due to differential contractile forces acting in the epithelium at a time when the wing is contracting from an inflated stage. This contraction has the effect of concentrating the epithelium cells within a smaller wing area. There is, however, no appreciable loss of cells. It is therefore of interest to estimate the number of cells in

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the shortened wings of the flies operated on at 5 hr. As each epithelial cell bears only one hair (Dobzhansky, 1929), this can be done by counting the number of hairs/unit area, and by measuring the total wing area. The cell count was made near the wing margin between  $L_4$  and  $L_5$  in a place where any dumpy effect might be expected to be operative. The total area was measured with a planimeter from a camera lucida tracing. The results are expressed in Table 1, but they can only be regarded as approximate since only a comparatively small area was



Text-fig. 10. Normal wing showing the conventions employed in this paper.

Table 1

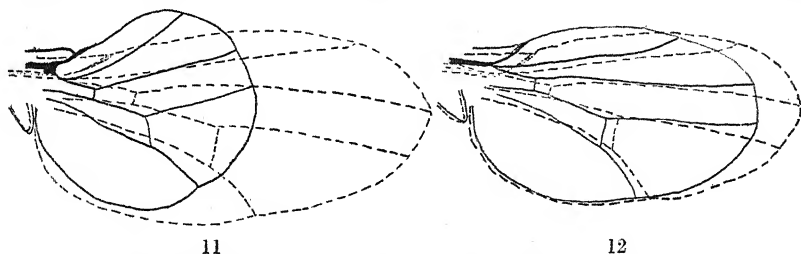
Hairs/0.01 sq. mm.	Total wing area in sq. mm.	Total hairs expressed to nearest 100
(1) Operated 62	1.10	6800
Control 58	1.37	8000
(2) Operated 62	1.13	7000
Control 52	1.77	9200
(3) Operated 55	1.47	8100
Control 52	1.70	8800

examined; also it was assumed for the purposes of the estimation that the hair density was similar over the whole wing surface.

It can be seen that in each example, although each cell does occupy a somewhat smaller area (about 10%), this does not account for the whole decrease in wing area. Evidently there has either been a degeneration of cells, or the full complement of cells has never been produced. Now at about 5 hr. the two wing surfaces have come together as a flat plate which is becoming longer through cell rearrangement and cell division. It seems reasonable to suppose that a wound in the epithelium has, at this time, the result of inhibiting cell division. Since this effect is easily

reproducible, it is possible to study its development in the operated pupae. Operated wings fixed at 8 hr. (i.e. before the dumpy contraction) are seen to be already considerably shorter and rounder than the controls; moreover, they more rapidly pass into the next stage of development, that of wing inflation, when the surfaces begin to be forced apart. This can be seen in Pl. 7, fig. 14, where the operated wing is considerably distended, while the control side is still plate-like. It would seem that, once the inflation has started, cell division is held in abeyance.

The dumpy phenomenon, which in the *dumpy* mutants produces its effect by altering the relationship of the forces resident in the epithelium and the veins, is also in evidence. Thus we have seen that the cell size is 10 % less. Another aspect is the splaying out of the veins, the maximum amount of distortion taking place peripherally. This can be demonstrated with reference to Text-fig. 10. For instance, in one example



Text-figs. 11, 12. Shortened and broadened wings resulting from 5 hr. operations, superimposed on the controls (dashed lines).

$\angle \theta$  and  $\angle \psi$  in the control are  $26^\circ$  and  $35^\circ$ , while in the operated wing they are  $33^\circ$  and  $53^\circ$  respectively. This can be more readily realized if the wings are superimposed (Text-figs. 11, 12). Correlated with this is the increase in breadth.

However, in these examples the dumpy process seems to work in a slightly different way to that of the mutants. In only a few wings (Pl. 7, fig. 15) is the contraction in one part of the margin stronger than that at other parts, the dumpy forces seeming to be more evenly distributed than in the mutants. Many small lax wings are produced; in these the wing membrane has the appearance of having contracted more longitudinally than the veins. Since the venation is very rarely disrupted (two wings show tilt effects of  $L_3$ ), one must suppose that all the original vein material is present; the veins must therefore be capable of telescoping. This can be well seen on the distal part of  $L_3$  which normally bears three or four chordotonal organs. In the shortened 5 hr. wings these are pushed considerably closer together.

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There is another possibility, namely, that the pronounced broadening effect on the wing contours may not be due to a dumpy contraction, but is somehow actively produced by a change in the shape of the epithelial cells. Now the hairs on the wing run for short distances in straight lines before merging with other rows of hairs. Convenient lines were therefore selected between  $L_3$  and  $L_4$  so that they ran approximately parallel, and at right angles to the length of the wing. By counting the number of hairs/unit distance along these two directions it was possible to arrive at an estimate of the cell shape. It was found that in the controls the sides of the cells, considered as rectangles, were expressed by the ratio 1 : 1.2, the longest side lying in the longitudinal axis of the wing; in the broadened wings this relationship was 1 : 1.4. In fact the cells of the controls were more nearly square than in the operated wings. Clearly therefore the whole of the broadening effect must be due to the dumpy contraction which causes the cells to slide over one another; this packs the cells closer together in a transverse direction, and actually produces a certain amount of narrowing of the cells.

The shape of these wings is therefore moulded first by a failure of cell multiplication at about 6 hr., and secondly by a dumpy contraction at about 22 hr., which is not so localized as in the *dumpy* mutants, but which is certainly more pronounced distally than proximally.

In most of the 5 hr. operations the venation is little affected, with the important exception of  $L_2$ . In two cases the base of  $L_2$  joins  $L_3$  distal to the anterior cross-vein (Pl. 7, fig. 16). Now it has been shown that  $L_2$  develops in a totally different manner to the other veins. The latter are represented in the prepupa, and are formed from cavities which never close when the wing surfaces come together.  $L_2$ , on the other hand, is built up from the fusion of small lacunae at the base of the epithelial cells which are present after contraction from the bag stage. It has no prepupal representative. It seems reasonable to suppose that the original wound in these examples was in the region of the anterior cross-vein, and that this prevented the formation of  $L_2$  proximally. The conditions regulating its origin might confer on  $L_2$  a greater degree of indeterminacy than the other veins.

### C. *Operations on 7-27 hr. pupae*

At about 7 hr. the two wing surfaces start to be forced apart by the pressure of the haemolymph. The wing continues to become more inflated until about 19 hr., when the process has reached its maximum and the

wing has become almost cylindrical. These events are reflected in the results produced by these operations.

During this period pricking results in large holes in the wing, and these indicate in a rough way the tensions present in the various parts. Since the operations were made by a circular needle, the original wound must have been circular. However, the holes in the imaginal wing are always longer than they are broad, indicating a greater tension along the wing than around the circumference. It has been pointed out by Waddington as an explanation for some dumpy phenomena that the degree of contraction might be related to the radius of curvature of the swollen wing, and that therefore a thin wing would have a smaller radius of curvature, and would tend to elongate farther. This seems to be borne out in these experiments. In general, holes at the base of the wing have the ratio length/breadth considerably larger than holes in the centre of the wing. Thus in two 8 hr. wings the ratios were 5 and 7, while in an example with a more distal hole it was 3. This does not actually seem to be due to the containing effect of the veins which are nearer together at the base, as there is often a certain amount of intervening membrane. In 18 and 21 hr. wings the tension of the epithelium has increased considerably; operations may leave the margin entire with an enormous central hole which is nearly circular. Such cases show that the stresses are disposed more or less evenly, as round the surface of a spherical balloon (Pl. 7, figs. 20, 21).

Exceptions to this type of wound are provided by 14-16 hr. wings in which an entire wing surface is produced, the site of the operation being marked by rather sparse hairs. The explanation may lie in the fact that from 7 to 13 hr. the wing is secreting a chitinous covering which is not thick enough to support the inflated wing if pricked. At about 16 hr. this is completed, and the capsule is firm enough to support the epithelium, and to allow regeneration to take place. Later the wing contracts from its sheath, and at these stages large holes are produced by pricking the inflated membrane.

These operations give information about (a) the process of dumpy contraction, (b) the method of vein formation, and the time of determination of (c) the chordotonal organs and (d) the cross-veins. These will be considered in turn.

(a) *Dumpy contraction.*

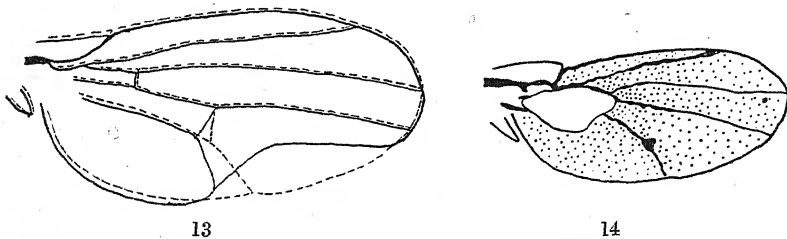
A typical *dumpy* wing exhibits a concavity in some part of the distal margin accompanied by a crowding of the cells. This may be due either



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to a weakness of the margin which is pulled in by the tensile strength of the epithelium, or to a contractility of the epithelium in excess of the normal. Both these processes seem to occur in these experiments. To consider the former process, if, for instance, the margin is removed anterior to the distal end of  $L_5$ , the latter is bent backwards towards the base. In one example  $\angle \theta = 33^\circ$  and  $\angle \psi = 40^\circ$ , compared with  $30^\circ$  and  $35^\circ$  in the control (Text-fig. 13). On the other hand, if portions are removed posterior to the tip of  $L_5$ , this is bent anteriorly, and the longitudinal veins are in general shifted slightly closer together relative to the membrane. Such a wing was measured and  $\angle \theta = 28^\circ$ , and  $\angle \psi = 33^\circ$ .

Defects in the anterior margin lead to shrinkage of the membrane away from the wound. This tissue movement is evidently hindered by the longitudinal veins which act to a certain extent as stiffening rods; these



Text-fig. 13. A 12 hr. operation showing the *dumpy* effect of marginal injury.

Text-fig. 14. A 21 hr. operation resulting in a *dumpy* contraction of the cells adjacent to the wound. The dots give an impression of the cell spacing.

may, however, be considerably bent by forces acting at right angles to them, and they may be forced nearer the next longitudinal vein. For instance,  $L_2$  may be nearer  $L_3$  (Pl. 7, fig. 17). In such a case it was found that the decrease in the total area between the veins was almost exactly compensated by the decrease in the cell size which was in the neighbourhood of 20%. In fact, this is a true *dumpy* phenomenon which, at least in part, can be seen to be due to a weakening of the supporting power of a margin normally counteracting the epithelial tension.

Examples also occur in these operations where the whole periphery has evidently lost its rigidity (Pl. 7, fig. 18); this results in wings of a very reduced size with crowded cells, and generally a very lax appearance. The thickening of the veins which invariably accompanies this state is described in the next section. There are, moreover, wings which are transitional between those with epithelial cells of a normal size, and those

with very small cells over the whole surface. In these wings the crowded cells are restricted to the neighbourhood of the wound and they increase in area away from it until at the periphery they are of normal size (Text-fig. 14; Pl. 7, fig. 19). It may well be that this dumpy phenomenon is of a rather different nature to that produced by removing the support of the margin, for in these transitional cases it is evident that the injury has induced an increased contraction of the epithelium rather than causing the mere release of a latent power of contractility by the destruction of the supporting members. If this is so, the production of a *dumpy* mutant wing may involve two separate processes, namely, a decreased resistance by the margin and an increased contractility of the epithelial cells.

(b) *Vein formation.*

A very characteristic effect of operations on wings 11–21 hr. old is the production of extra vein material. In some cases this may be more or less regular in appearance, for instance, a cross-vein may be intercalated between  $L_2$  and  $L_3$ . More generally the chitinization is irregular as in the mutant *plexus*; it is sometimes related to the extent of the wound. Now it has been shown that the formation of a vein depends primarily on the maintenance of an open lumen between the two wing surfaces (Waddington, 1940). Epithelial cells lie in a denser, thicker layer over these cavities than over the intervein area. In several mutants, such as *net* and *plexus*, extra chitin is produced in a different way, more like that which normally takes place with regard to  $L_2$ , namely, by the fusion of small lacunae. It is not clear which process is the most important in the operated wings, but it is quite definite that the effect is intimately connected with the incomplete juxtaposition of the two surfaces. In 18 and 21 hr. wings possessing a large central hole, this latter is often lined by thick vein material, while veins whose proximal ends are removed have striking thickenings where they join the hole. These swellings are triangular, and bear a remarkable resemblance to the terminal vein thickenings of the mutant *Delta* (Pl. 7, figs. 20, 21).

Related in origin to these wings is another group in which the *Delta* effect is not localized. These are the small, dumpy wings mentioned in the last section. Pricking has not had the effect of causing a powerful tissue contraction away from the wound, but has resulted in a *generalized* dumpy contraction over the whole wing surface; this often has had the effect of virtually closing the holes made by the operations. In these wings all the veins have a thickened, beaded appearance (Pl. 7, fig. 18).

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In *dumpy* mutant wings the veins may be considerably foreshortened, but the final thickness of the veins is similar to that of the normal wing. In these operated wings the concentration of vein material owing to telescoping is evidently not offset by any such compensating process, and they become thick. This appearance may be exaggerated by a phenomenon which takes place in *Delta* wings, namely, an absence of the vein thinning normally taking place at about 30 hr. Finally, the condition of the veins in the transitional wings, where the small, dumpy cells are restricted to the region of the wound, is very striking. Here the thickened vein material is only found in the area of the dumpy contraction; outside this area the veins are normal (Text-fig. 14).

### (c) *The chordotonal organs.*

It seems probable that the presence or absence of chordotonal organs on  $L_3$  is not fixed at the time of pupation. Out of seven 0 hr. wings with pronounced tilt effects on  $L_3$ , none bear these organs distal to the missing section of vein; they are, however, always present proximally to the defect, which suggests that at this stage the chordotonal organs are dependent on the integrity of the nerve running down  $L_3$  (Text-fig. 8). In one wing, with a slight weakening of the base of  $L_3$ , one chordotonal organ was present distally. However, this was not a characteristic tilt effect, and it seems possible that the nerve might have been relatively undamaged.

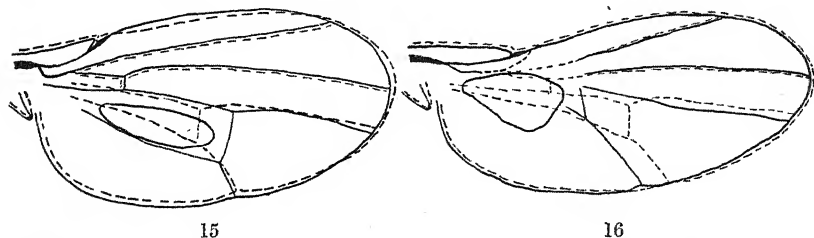
Two 5 hr. tilts exhibit one chordotonal organ distal to the injury. A section of vein of this length would normally bear two or three such organs. Operations of 11 hr. and subsequently may result in large holes disrupting the base of  $L_3$ . In all these cases the full complement of chordotonal organs is present on the distal section (Text-fig. 9).

When  $L_3$  bifurcates, as in some 0 hr. operations, and the main branch joins  $L_4$ , the combined material of these two veins is also capable of giving rise to chordotonal organs, although  $L_4$  normally never bears them. In such a case the nerve of  $L_3$  is probably diverted together with the overlying epithelium, and this is capable of influencing the combined vein material. (Cf. Pl. 7, fig. 4.)

Probably the determination of the chordotonal organs takes place around the 5 hr. stage, but the final number of organs produced may be dependent on such factors as the degree of destruction of vein material, the length of the nerve in the remaining section, etc.

(d) *The cross-veins.*

These experiments give certain information about the time of determination of the cross-veins. Neither of these has a visible prepupal representative. Waddington has shown that the position of the posterior cross-vein is probably not fixed until the end of contraction from the bag stage, when this vein is all that remains of the central vesicle. In the mutant *veinlet*, where the distal ends of  $L_3$ ,  $L_4$  and  $L_5$  disappear, the posterior end of the cross-vein may meet the tip of  $L_5$  at a very conspicuous slant. The process, however, is very variable, and seems to depend on the time relations of the disappearance of  $L_5$ , and the formation of the cross-vein. In operations from 8 to 21 hr., the posterior cross-vein is often pushed towards the periphery if the wound is proximal to it (Text-fig. 15). In one control the area of the distal region *GCEF* (Text-fig. 10) measured 1.5 times that of the operated wing. There was some



Text-figs. 15, 16. 18 hr. operations resulting in alteration of the position of the posterior cross-vein.

dumpy contraction in the latter, but the cell number/unit area was only 1.3 times greater than in the control. Conversely, some 21 hr. operations, which give rise to a small hole near the base of the wing, have the effect of shifting the cross-vein in the opposite direction. Thus in one example (Text-fig. 16) the region *GCEF* was increased in area by about a quarter while the cell size was normal. It would seem therefore that the cross-veins in these operated wings were formed from cells which would normally produce intervein material. Final determination may take place at a slightly later date, after the contraction of the central vesicle. Abundant convincing examples are not to hand, as defects in the inflated wing usually inhibit the coming together of the two epithelia; under these conditions the cross-veins are often completely absent. In many cases the posterior cross-vein is malformed, and occasionally there are the rudiments of two such veins.

The anterior cross-vein rarely shows such irregularities. Morphologically it seems to be part of  $L_3$  as it bears a chordotonal organ centrally.

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These organs only occur on  $L_3$  and the base of  $L_1$ . In operations where the cross-vein is absent, and in the mutant *cross-veinless*, the chordotonal organ is present on the membrane between  $L_3$  and  $L_4$ . Evidently in *cross-veinless* the nerve branch to the anterior cross-vein is intact, but this does not necessarily lead to vein formation.

In several 0-3½ hr. wings the distal half is completely missing. Under these conditions the anterior cross-vein may be situated more basally than in the controls. The region *ADIH* may be only two-thirds of its normal area; this, however, is completely compensated by the decrease in cell size.

In operations from 7 to 21 hr., which produce holes in the cell *ADIH*, the cross-vein may be moved in the opposite direction. The decrease in the length of the cell *ABCD* is usually accompanied by an increase in breadth so that the total area remains the same. Moreover, the hair density is similar. There is no reason to suppose, therefore, that certain epithelial cells have not by this time been determined to form the anterior cross-vein, if given the right conditions for vein formation. The isolated chordotonal organ may be the only sign of such a determination if the conditions are not favourable.

#### 4. DISCUSSION

Although the operations which have been performed on the wing buds are only the simplest kind of defect experiments, they have given results which are of sufficiently numerous kinds, and which occur in sufficiently regular correlation with the time of operation, for several important conclusions to emerge.

We may first consider the determination of the veins. These may be taken in groups: the margin;  $L_3$ ,  $L_4$  and  $L_5$ ;  $L_2$ ; the anterior cross-vein, and the posterior cross-vein. The first point which strikes one is that in wings operated at 0-3½ hr. remarkably few of the operations produce any visible result at all, and in those which have had any effect, it is always the margin which is injured and never the main surface of the wing. These two facts taken together show clearly that the main blade of the wing is still capable of a considerable amount of repair at this time, while the margin is capable of much less. In fact, since there is as yet no positive evidence of any kind pointing to regulation of the margin occurring after pupation, it is simplest to take it provisionally that the margin is completely determined at that time.

The longitudinal veins cannot be completely determined at the time of pupation, since, as we have seen, the main blade of the wing, in which

they are included, is still capable of regeneration. On the other hand, the operations show that their position is already in some way foreshadowed, since in wings which have lost part of the margin, the veins are not regulated to fit smoothly into the new wing outline, but remain in their normal arrangement, so that the wings appear as if pieces had been cut away from the edge of a normal wing. This is the characteristic appearance of a wing produced by the "scalloping" genes, such as *cut*, *vestigial*, etc. The production of phenocopies of these mutants by operations at the time of pupation is evidence, though not in itself conclusive evidence, in favour of the view advanced by Waddington (1940) that these genes act by modifying the folding of the wing area at this time and not by a later degeneration as suggested by Goldschmidt (1935, 1937). The conclusion is strongly supported by the fact that the percentage of scalloped phenocopies declines to zero after the complete invagination of the wing-fold. The series of deductions made by Waddington from the study of the development of these mutants have in fact received confirmation at every point at which they can be tested. Apart from the hypothesis, which we have just seen to be true, that the course of the longitudinal veins is, at the time of pupation, no longer affected by changes in the shape of the margin, it was suggested that the veins must be to some extent labile in that, however abnormal the folding of the wing area, vein material which should appear on the dorsal surface of the wing never develops as vein on the ventral surface or vice versa. This incompleteness in the determination of the vein material can, in the first place, be deduced from the same type of evidence in the operated wings. It may be, in fact, and probably is, that the scallopings in the operated wings are produced in the same way as in the mutants, by an alteration in the folding, and in that case the same arguments as to the failure of vein material to differentiate on the wrong surface will apply. But even if we suppose that the operative scallopings are caused by mechanical removal of part of the margin, it is difficult to escape the same conclusion. It is to be noticed that even the edge of the scalloped regions is double-layered; we do not find, except in very rare cases, that the dorsal epithelium extends farther than the ventral, for example. Since it is difficult to believe that the needle has always passed exactly perpendicularly through the wing and injured the two surfaces exactly symmetrically, this can only mean that after the injury the cut edges join up and make a single fold with all the material still available. Whenever the injury was asymmetrical part of that wing surface which was left with an excess of material must therefore eventually pass over into the other relatively

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diminished surface; and if the veins were fully determined, we should again expect to find part of the vein developing on the wrong surface. Since we do not in fact do so, we must conclude that the veins are only partially determined and are abolished if they are folded on to the wrong surface.

Perhaps more immediate evidence of the relative nature of the vein determination is the fact, mentioned above, that regeneration of the wing surface is possible after injuries to the central portions of it. Further confirmation appears to be provided by the cases in which the venation is partly disorganized. In these we frequently find cases in which veins branch or form loops. If this were due merely to the mechanical separation of already determined parts there is no reason why similar results should not be produced by later operations; and the fact that they are not produced there strongly favours the alternative explanation, that the branchings and loopings are caused by the relative shifting of the upper and lower epithelia at a time when the process of vein determination is still proceeding. Such shiftings of the epithelia can be certainly demonstrated by reference to the irregular hair distributions which were referred to above (p. 121).

In some of these vein deformations it can be seen that veins which occupy an abnormal position have typical vein material of both their upper and lower surfaces. It seems impossible to explain this on the hypothesis of a mechanical disturbance of determined material, since it is in the highest degree improbable that upper and lower epithelia would be deformed in exactly the same way; and, moreover, one can see from the hair distributions that they have not been. Finally, the evidence of the faint vein occupying the normal position makes this supposition unnecessary. One is forced to the conclusion that vein material on one epithelium has, after being shifted to a new position, been able to induce the material in the opposite epithelium to form vein surface. It is evident that the period of the induction process coincides with the only time in the pupal history of the wing when the two surfaces can be shifted relatively to one another. An inductive relation of this kind was deduced by Waddington from the study of the development of the scalloped mutants; it seems here to have received complete confirmation from quite a different type of evidence. The present data, furthermore, indicate that the dorsal epithelium is probably the inducing surface, a conclusion which agrees with the morphological grounds advanced in Waddington's paper.

Presumptive vein material of the inducing epithelium can, then, be

prevented from developing as vein if it passes over into the other surface. Apart from this, there are two other phenomena in which the normal venation fails to develop. The first of these is seen in the phenocopies of *fused*. No complete study is yet available of the development of fused-like wings, either for the mutant race or the operated flies, but the effect is exactly what one would expect if the prepupal arrangement of  $L_3$  and  $L_4$  persisted into the imago, and it is mostly simply interpreted as an inhibition of development. There is no need to suppose that it involves the suppression of vein differentiation by any of the presumptive vein material.

Such a suppression of histological development seems to occur in the tilt group of wings. Its exact causation is obscure, since the effect is not always confined to the actual site of injury. It cannot be said with any exactitude whether injuries confined entirely to intervein epithelium can cause vein suppression, but it is quite definite that a localized injury to a vein ( $L_3$ ) may cause its complete disappearance for some distance both distally and proximally to the wound. A somewhat similar, and even more widespread, inhibition of vein development was described by Henke (1933) as a result of defect experiments on the imaginal buds in Lepidopteran larvae. It is to be noted that this inhibition of histological differentiation can occur considerably after the final determination of the position of the veins has taken place, since it has been found in wings operated as late as 6 hr. after puparium formation. At still later periods the longitudinal veins appear to be completely determined against all the agencies which have been brought to bear on them. They are, of course, still subject to mechanical distortion, such as that due to abnormal contraction.

The other veins remain in a labile condition until later; perhaps the difference is not so much in the actual biochemical state of the vein material as in the kind of influences which can be brought to bear on it by the operative means at present at our disposal. At any rate, it is found that  $L_2$ , which has no prepupal representative, and which forms after the contraction by the fusion of small spaces, can be shifted by operations made as late as 5-6 hr. after puparium formation. As for the posterior cross-vein, it had previously been deduced from morphological evidence that it remains labile almost until it begins to be visible after the contraction, and this has now been experimentally confirmed; the vein can be moved by operations as late as 21 hr. There is not sufficient evidence to fix the time of determination of the anterior cross-vein.

The histological fate of the intervein membrane cannot be fixed until



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fairly late in development. Thus a shifting of the posterior cross-vein would not be possible if the intervein material were already determined. Similarly, it is found that extra vein-like material may be formed as a consequence of late operations. It may take the form of plexus-like fragments of vein; their formation seems to be due to a fusion and enlargement of the spaces between the bases of the cells, much as occurs normally in the development of  $L_2$ . Extra vein material also often forms along the edges of wounds. It seems that here the vein-like differentiation is caused by the fusion of the cut edges of the dorsal and ventral epithelia to give a fold which encloses a hollow space. The causal relation between vein differentiation and the presence of a space within the wing is obscure and would repay study. It seems that vein-like material always has a space beneath it, but the relation is not reversible, since not all the epithelium covering spaces becomes vein-like. In many cases this failure may be accounted for by the fact that the space has been produced by an event very late in development, such as an abnormality in the flattening and drying out of the wing after emergence, but this explanation cannot hold, for instance, for the wings of the mutant *bloated*, in which the abnormal vesiculation can be seen soon after the contraction stages.

The last elements in the venation which need consideration are the chordotonal organs. No mutations affecting these have been studied, and nothing was previously known of their development. In the operated wings it is clear that if  $L_3$  is interrupted before about 5 hr. no chordotonal organs develop distal to the injury, whereas in later stages they occur on both sides of the injured section. It is apparent that their determination takes place at about 5 hr., and is dependent on some influence which passes down  $L_3$  from the base distally. The nerve and tracheole which grow down this vein would exactly fill the role of the inducing stimulus, and it is probably reasonable to look on one or other of them as responsible, but no direct confirmation of this has been obtained, since they are both invisible in adult wings.

The appearance of small rounded wings after operations at about 5 hr. is very characteristic, but a definitive explanation of it is not easy to provide. It is clear that one of the primary factors is a reduction in the rate of cell division. It was shown (Waddington, 1940) that in some mutant types, such as *broad*, *narrow*, etc., the alterations in wing shape are brought about by alterations in the relative rates of cell division in directions, and it is very probable that a mere reduction in division rate would have some effect on shape through a dislocation of the normal growth rates. Moreover, it is also known that if a wing is, before the

contraction stage, broader than usual (as in *Xasta*, for example), the contraction acts in such a way as to increase its divergence from normal. It may be that these two factors are sufficient to explain the roundness of the operated wings. The 5 hr. wing is also undergoing a process of elongation by rearrangement of its cells, and some inhibition of this may also play a part.

Finally, many of the operations, particularly the later ones, provide clear evidence of the reality of the phenomenon which has been spoken of as the dumpy contraction. Moreover, the character of the distortions produced shows that, as was previously suggested, the contracting element is the epithelium, while the veins and margin act rather as stiffening rods which oppose the contractile forces. There is a possibility, however, that the dumpy contraction may involve two components. First, a weakening of the resistance of the margin would cause the normal tension of the epithelium to draw it in. Secondly, certain factors (such as some injuries at about 20 hr.) cause an *increase* in the normal tension of the epithelium which may be local or spread over the whole wing surface. In the *dumpy* mutants either or both of these processes may be operative.

## 5. SUMMARY

Defect experiments on the pupal wing of *Drosophila melanogaster* are described. Appreciable effects are only produced from 0 to 25 hr. after pupation at 25° C. The injured wings may be classed under three chronological headings.

### A. 0-3½ hr.

(a) *Scalloping effects* where part of the margin is missing. These occur in only 5 % of the operated wings, and are probably caused indirectly by altering the position of the wing-fold during invagination. It follows that the margin is completely determined at this stage, and that the veins are at least in part determined since there is no sign of accommodation to fit the new size of the wing surface. The veins may, however, be abolished if folded on to the wrong surface.

(b) *Disruption of the venation*. This defect involves a branching of  $L_3$ ,  $L_4$  and  $L_5$ . The disturbed vein is usually as thick as a normal vein, while the subsidiary branch is thin and usually occupies the normal position. The hair distribution shows that the dorsal surface has been moved laterally relative to the ventral surface. This is direct evidence of an induction process by the dorsal epithelium. The faint ventral vein is a remnant of the half-completed process. Similar conclusions may be

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arrived at indirectly by a consideration of the scalloped mutants or phenocopies.

(c) *Fused effects*. In these wings the bases of  $L_3$  and  $L_4$  are confluent. This may be the result of the retention of the normal prepupal dichotomy.

(d) *Tilt effects*. These involves the complete absence of a section of vein as in the mutant *tilt*. The commonest vein to be effected is  $L_3$ , and this may be related to the presence of the nerve running down it. The inhibition is not confined to the exact site of the injury.

(e) *Vesiculation effects*. Wings of this category exhibit large blisters resembling those of the mutant *vesiculated*. These are probably caused by a failure of the wing surfaces to adjust themselves after contraction from the inflated stage.

### B. $3\frac{1}{2}$ –6 hr.

Operations at this period produce very characteristic round, short wings. They are the resultant of two main abnormal processes. First, an inhibition of cell multiplication which normally takes place at this time; this upsets the differential growth rates of the cells. Secondly, an occurrence of the dumpy process affecting the contraction from the inflated stage. This tends to make broad wings broader still, and splays out the veins.

The exact course of  $L_2$ , which is formed in a different way to the other longitudinal veins, can be modified up to this time.

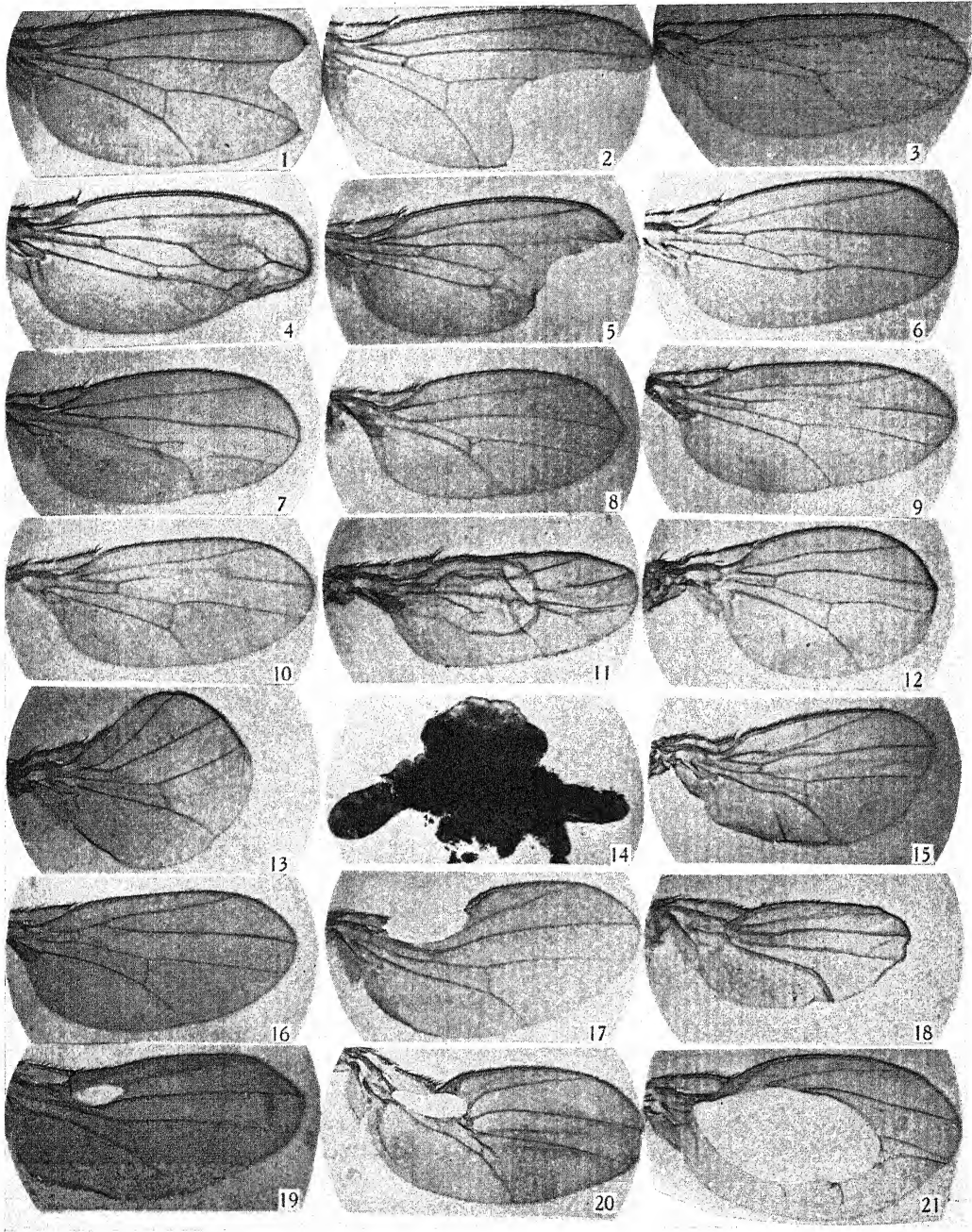
### C. 6–21 hr.

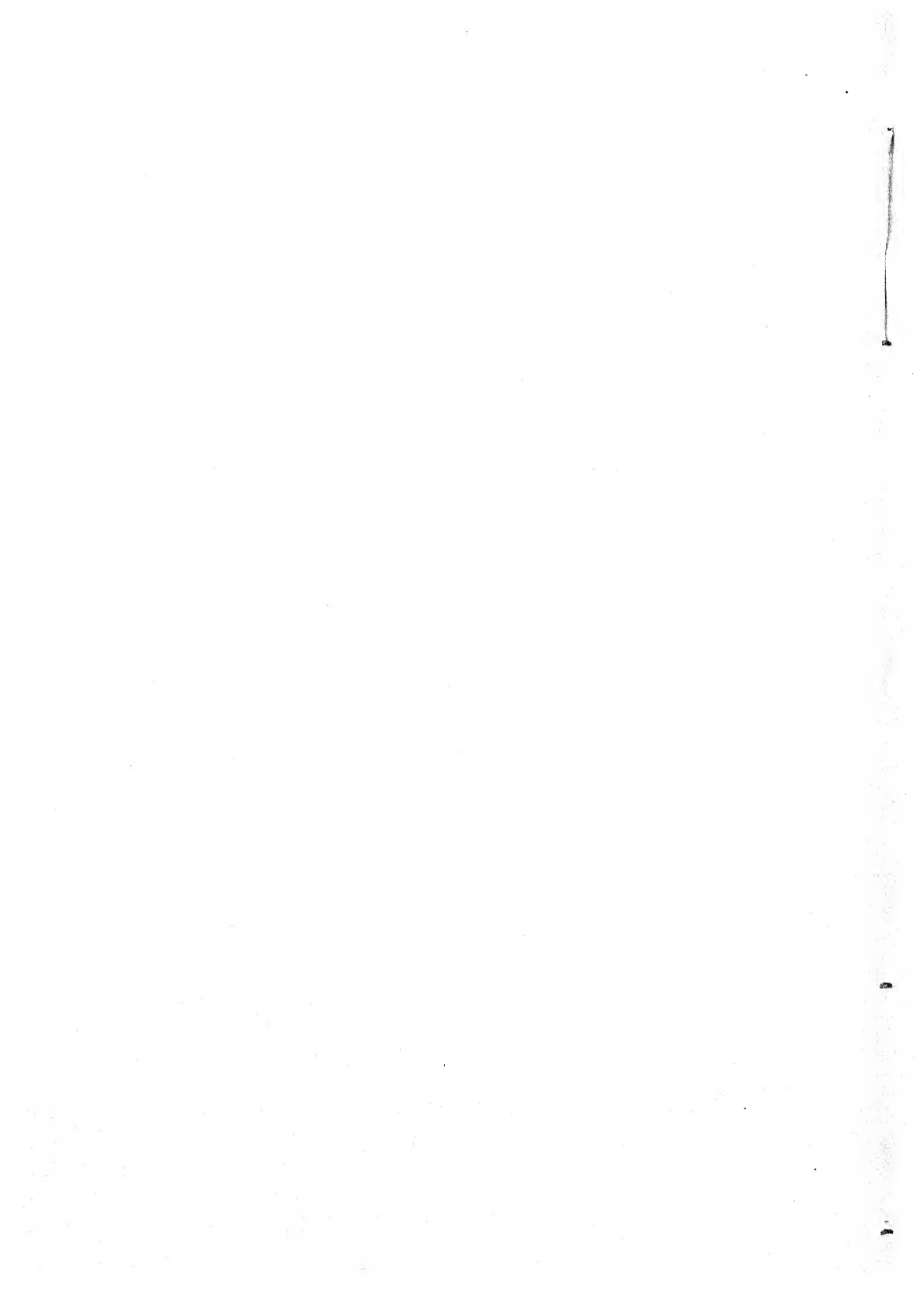
(a) Injuries at this time when the wing is very inflated give rise to large holes in the epithelium, and afford an opportunity to examine the dumpy process in detail. This is found to be due to two main causes: first, a decrease in the marginal resistance, and secondly, an increase in the epithelial tension. These factors may result in a distortion of the wing outline, and a local or widespread crowding of the cells.

(b) Extra vein material such as is found in the mutant *Delta* frequently occurs near wounds made at this time.

(c) In 0 hr. tilt wings where  $L_3$  is broken, chordotonal organs are never found distal to the injury. The converse is true of operations later than 5 hr. It is suggested therefore that these organs are determined at about this time, and that their differentiation is dependent on the integrity of the nerve innervating them.

(d) The position of the posterior cross-vein can be altered as late as 21 hr., and can be shown to be produced from cells that would normally





give rise to intervein membrane. There is no evidence to show that the anterior cross-vein can be similarly modified.

I should like to express to Dr C. H. Waddington my thanks for the suggestion of this problem, also for his many helpful criticisms throughout the course of this work, which was carried out during the tenure of a grant from the Department of Scientific and Industrial Research.

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## EXPLANATION OF PLATE 7

All photographs are  $\times 20$ .

- Figs. 1, 2. Operated 0 hr. Scalloping effects.
- Figs. 3-7. Operated 0 hr. Disruption of the venation.
- Fig. 8. Operated 0 hr. *Fused* effect.
- Figs. 9-10. Operated 0 hr. *Tilt* effects.
- Fig. 11. Operated 0 hr. Vesiculation effect.
- Figs. 12, 13. Operated 5 hr. Broad, *dumpy* wings.
- Fig. 14. Operated 5 hr., fixed 8 hr. The plate-like left wing is the control. Note the sac-like right wing.
- Fig. 15. Operated 5 hr. *Dumpy* wing showing marginal distortion.
- Fig. 16. Operated 6 hr.  $L_2$  joins  $L_3$  distal to the anterior cross-vein.
- Fig. 17. Operated 18 hr. Local *dumpy* effect due to the absence of the anterior margin.
- Fig. 18. Operated 18 hr. Widespread *dumpy* effect due to increased epithelial tension and collapse of the margin.
- Fig. 19. Operated 18 hr. Local *dumpy* effect due to increased epithelial tension near the wound.
- Figs. 20, 21. Operated 21 hr. *Delta* effects.

## ADDENDUM

Since this paper went to press, work by Braun (*J. exp. Zool.* **84**, 325-49) has appeared dealing with the effects of defect experiments on the pupal wings of several *melanogaster* mutants. The operations were performed with an electric cautery. The mutant stocks used were *vestigial-notched*, *cut*, *net*, *plexus* and *Plexate*.

Operations on 12-24 hr. *vestigial-notched* pupae did not produce, as might have been expected from Goldschmidt's hypothesis of diffusing lytic substances, any modification in the pattern of scalloping. There was, however, a change in shape of some of the wings which may, in the light of the present results, be ascribed to dumpy contractions in the epithelia. Alterations in the position of the posterior cross-vein are also recorded. Braun also figures six wings of *vg<sup>no</sup>* pupae wounded at 36 hr. which show extra scalloping. In the present experiments no scalloped wings resulted from operations as late as 36 hr.; indeed the only visible results were very small defects in the membrane or margin. It is extremely difficult to understand how pricking could, as this stage when most of the definitive wing characters are differentiated, produce such a considerable and widespread effect. According to the evidence presented in this and in Waddington's papers, characteristic scalloping is produced during the infolding of the wing epithelium at about the time of puparium formation. It would seem, therefore, that the extra scalloping of the *vg<sup>no</sup>* must be due to genetic rather than operational causes.

The most interesting effect described in Braun's paper concerns the orderly reduction in the amount of extra venation in mutants such as *net* and *plexus* as the result of operations from 12 to 24 hr. Possibly the primary effect of injury at this time is one of partially deflating the normally swollen wing, thus closing some of the cavities whose maintenance is a necessary adjunct of vein formation. It is noticeable that the effects of the operations are not confined to the exact site of the injury.

The bulk of the present work deals with the effect produced by operations carried out before 12 hr., which is the earliest time at which Braun's were made. However, precise comparison is difficult, since the temperature at which Braun's pupae were incubated is not recorded. Furthermore, the pupal age is frequently only given to within 12 hr. This is a period of time embracing, especially during the early pupal instar, very varied embryological and histological changes which are reflected in the results of the operations. These are, however, quite characteristic of the exact time at which they are made.

# THE EVOLUTION OF THE SEX CHROMOSOMES

## I. THE XO AND $X_1X_2Y$ MECHANISMS IN PRAYING MANTIDS

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(With Plates 8-13 and 12 Text-figures)

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### 1. INTRODUCTION

THE evolution of the sex-determining mechanism is one of the most interesting chapters of chromosomal evolution, but the phylogenetic history of the sex chromosomes can only be interpreted when the mechanism of meiosis is understood. In some groups of organisms the sex chromosomes differ very little from the other chromosomes, undergoing pairing and segregation at meiosis in much the same way as the autosomes. In other groups of animals the meiotic mechanism in the heterogametic sex has been highly modified so far as the sex chromosomes are concerned, so that they behave in an entirely different manner from



the autosomes. It is possible to draw a primary distinction between groups (such as the Mammalia and the Diptera) in which chiasma formation and crossing-over take place between the  $X$  and  $Y$  chromosomes and groups (such as the Neuroptera, Heteroptera and possibly the Dermaptera) in which no interchange of materials can take place between the  $X$  and  $Y$  at meiosis. In the first two groups there is always a *pairing segment* which is common to both  $X$  and  $Y$ ; in the others it is not possible to recognize any pairing segment by cytological means.

In some groups of animals complex sex-chromosome mechanisms have been described in which more than one kind of  $X$  (or more than one kind of  $Y$ ) is present. It has recently been shown (White, 1940*b*) that these multiple sex-determining systems ( $X_1X_2Y$ ,  $X_1X_2X_3Y$ ,  $XY_1Y_2$ ,  $X_1X_2O$ , etc.) have arisen in a number of different ways from the simpler  $XY$  and  $XO$  mechanisms and that their origin and evolution in particular cases can only be interpreted in relation to the meiotic mechanism which prevails in the particular group of organisms under consideration.

Previous work on the cytology of various species of Praying Mantids (Oguma, 1921; King, 1931; Asana, 1934) has demonstrated the existence of an  $X_1X_2Y$  ( $\delta$ ) :  $X_1X_1X_2X_2$  ( $\varphi$ ) mechanism in a number of genera, but these workers did not attempt to elucidate the origin of the mechanism. More recently the present author (White, 1938) studied a Mantid whose sex chromosomes were of the simple  $XO$  ( $\delta$ ) :  $XX$  ( $\varphi$ ) type. The present work was planned in order to clear up the whole evolutionary history of the  $X_1X_2Y$  system, so far as that was possible by studying the species still living at the present day. The work was unavoidably brought to a close by the outbreak of war, but a sufficiently large number of species were studied to enable general conclusions to be drawn.

## 2. MATERIAL AND METHODS

Altogether eleven species belonging to ten different genera and five subfamilies were examined:

Species	Subfamily	Country of origin
<i>Ameles abjecta</i> Cyr.	Amelinae	S. France
<i>Empusa egea</i> Charp.	Empusinae	"
<i>Gongylus gongyloides</i> L.	"	Ceylon
<i>Iris oratoria</i> L.	Photininae	S. France
<i>Acontiothespis</i> (= <i>Acontista</i> ) sp.	Acontistinae	Mexico
<i>Callimantis antillarum</i> Sauss.	Mantinae	Haiti
<i>Miomantis</i> (= <i>Calidomantis</i> ) sp.	"	S. Africa
<i>Sphodromantis</i> sp.	"	"
<i>Sphodromantis viridis</i> Forsk.	"	Algeria
<i>Paratenodera sinensis</i> Sauss.	"	U.S.A.
<i>Tenodera aridifolia</i> Stoll.	"	Malay States

The material of *Callimantis* and *Acontiothespis* was collected in the wild, the first by Dr K. W. Cooper, the second by myself. The other nine species were reared in the Department of Zoology, University College, London, from eggs sent through the post from abroad. The method of rearing was exceedingly simple. As is well known these insects deposit their eggs in a mass of foam which coagulates after a few hours into a hard but exceedingly light body known as the oötheca. As soon as a batch of oöthecae were received they were placed in half-pint milk bottles which were kept in a large cage with glass sides. This cage was kept at a temperature of 28–29° C. by means of an electric heater. This heater was controlled by a toluene-mercury regulator in a relay circuit. The eggs usually hatched out after a few weeks and the young nymphs were then isolated from one another, each one being placed in a separate milk bottle with a few twigs to climb up. All the milk bottles were kept in the same cage on shelves made of perforated metal, a small fan ensuring that the air was kept in circulation between the bottles.

The young nymphs were fed exclusively on *Drosophila*, the older ones and the adults on *Ephestia*, blow-flies, mealworms, grasshoppers and even (in the case of *Sphodromantis viridis* and *Gongylus*) young frogs. Most species of Mantids are cannibalistic, so that when an excess of a particular species hatched out they could be used as food for larger individuals. The smaller species were kept in half-pint bottles throughout their life, the medium-sized ones were later transferred to one-pint bottles, the very large ones to wooden cages with gauze sides. Using these very simple methods it would probably be possible to rear any species of Mantid in temperate climates. The only forms which are really difficult to rear are those in which the first instar nymphs are very small and weak so that they have great difficulty in catching *Drosophila*. I was never able to find a really satisfactory food for the very small nymphs of *Ameles*; *Drosophila* of some mutant stock such as *vestigial* which reduces the power of flight were the best kind of food, but were not entirely satisfactory. That the rearing methods were generally quite adequate is shown by the very low mortality (most of it accidental) and the fact that several species were reared through more than one generation (*Miomantis* gave two complete generations in six months).

The meiotic divisions in the testis generally take place in the last nymphal instar, the testes of the adults usually containing nothing but sperm. Younger nymphs frequently show spermatogonial divisions in their testes, but the meiotic divisions seem to be passed through very

rapidly. A good deal of material was wasted before this fact was realized.

The fixatives used were strong Flemming, San Felice, Navashin and Allen's Bouin. San Felice was found to be a very excellent fixative and was used in nearly all the later work. All the material was sectioned (at about  $16\mu$ ), since it was found difficult to make good smears. Nearly all the slides were stained in gentian violet by Newton's method (the staining period being frequently as long as 4-5 hr. after fixation in San Felice).

### 3. THE XO SPECIES

#### (a) *Ameles abjecta*

This is a small Mantid with wingless females which is a native of southern Europe. A number of oöthecae collected in the south of France were obtained from a dealer and several dozen adults were reared from the eggs.

The spermatogonial metaphases of this species show 29 chromosomes of which the largest is the mediocentric X-chromosome. This is the highest chromosome number so far recorded for any Mantid. The autosomes were all very small, and it was not possible to determine whether they are all mediocentric, or whether some of them may not be telocentric.<sup>1</sup> As is usual the X-chromosome is positively heteropycnotic in the "resting stages" between the spermatogonial divisions. At the first meiotic division fourteen autosomal bivalents are formed (Pl. 9, fig. 10). The behaviour of the X does not differ in any way from that seen in *Iris* and *Miomantis*, which were studied in greater detail than was possible in the case of *Ameles*.

#### (b) *Empusa egena*

This and the next species are members of the highly specialized subfamily Empusinae. The members of this group may be distinguished from all other Mantids by the peculiar extension of the vertex into a conical protuberance. Half a dozen nymphs of *Empusa egena* were obtained from a dealer and some of them were reared to maturity.

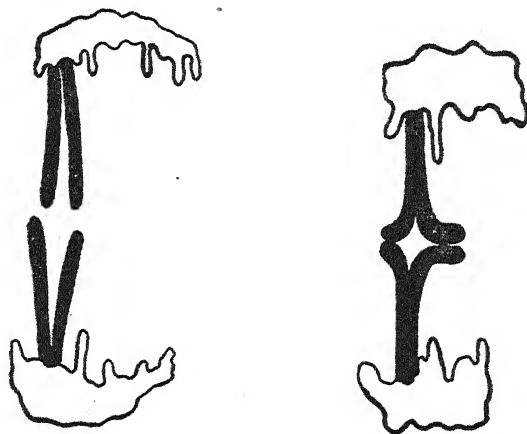
<sup>1</sup> The term telocentric will be used throughout this paper to indicate a chromosome in which the centromere is effectively terminal, so that the chromosome consists of one limb only (if a second limb is present it is so minute that for most purposes it can be disregarded). The term mediocentric indicates a chromosome in which the centromere is interstitial, so that the chromosome consists of two limbs, which may be either equal or unequal in length. This terminology seems to be more convenient than the use of the expressions "rod-shaped" and "V-shaped".

Spermatogonial metaphases of this species show 27 chromosomes (Pl. 8, fig. 3). All the autosomes are mediocentric, but in some cases one limb is much longer than the other. The *X* is by far the longest chromosome in the set, and has slightly unequal arms. It is positively heteropycnotic in the spermatogonial "resting stages" and throughout the prophase of the first meiotic division. No good preparations of the first metaphase were obtained in *Empusa*, since the animals were killed at the beginning of the last nymphal instar, when hardly any of the spermatocytes had developed beyond pachytene.

(c) *Gongylus gongyloides*

This species is a native of Ceylon, whence I received two oöthecae through the kindness of Dr H. M. Henry.

The spermatogonial chromosomes of *Gongylus* are shown in Pl. 8, fig. 4. The chromosome number is the same as in *Empusa*, namely, 27.



Text-fig. 1. *Gongylus*: spermatogonial anaphases. X-chromosomes in black.

All the autosomes are mediocentric and vary considerably in size. The *X* is an enormous mediocentric chromosome—even larger than in *Empusa*, both absolutely and relative to the length of the autosomes. In the cell shown in fig. 4 it is lying at the edge of the spindle, but it is frequently situated in the middle of the spindle, with its long arms lying above and below the equatorial plane. The spermatogonial anaphases and telophases of *Gongylus* have a very curious appearance (Text-fig. 1) owing to the fact that the ends of the *X* are still incompletely separated even when

all the other chromosomes have reached the poles. No observations were made on the meiotic divisions of this species.

(d) *Iris oratoria*

This species is a native of southern Europe. Numerous adults were reared from oöthecae obtained from a dealer.

The diploid number of this species is 25 in the male. It is possible that some of the chromosomes are telocentric, but no decisive observations were made. The larger autosomes and the X are in any case mediocentric (the X is seen in Pl. 9, fig. 12). The 12 meiotic bivalents are rather different in appearance from those of the other species studied, being much more elongated in an axial direction (Pl. 9, fig. 12).

(e) *Acontiothespis* sp.

A single unidentifiable nymph belonging to this genus was collected in Yucatan. It proved to have the lowest chromosome number hitherto recorded for any Mantid (15 in the male). All the chromosomes are mediocentric. The meiotic chromosomes are very large and clear, but not all stages were represented in the very scanty material available. A polar view of the first metaphase is shown in Pl. 10, fig. 13, while an idea of the structure of the meiotic chromosomes in side view can be obtained from Text-fig. 2 and Pl. 13, fig. 33.



Text-fig. 2. *Acontiothespis*: first metaphase. Only four bivalents and the X-chromosome are shown. In three of the bivalents the tearing apart process has been more or less completed, in the one on the right it has only just begun.

In general it may be said that this species approximates to the *Callimantis* type of meiosis. The bivalent on the right of Text-fig. 2 (which is the same one as that shown in Pl. 13, fig. 33) might in fact be a *Callimantis* bivalent. The other bivalents in this cell have, however, undergone a certain degree of stretching in the spindle so that the chromosomes of which they are composed have become torn apart.

This process, which in *Callimantis* happens at the anaphase of the first meiotic division, seems to occur somewhat earlier in *Acontiothespis*, so that most of the bivalents have undergone a certain amount of tearing apart during the early part of metaphase.

A relatively large granule frequently marks the position of the centromeres in the meiotic chromosomes of *Acontiothespis*. It is seen very clearly in the X-chromosome drawn in Text-fig. 2.

(f) *Callimantis antillarum*

The meiosis of this species has already been described in a former paper, so that all that is necessary is to compare it with the species subsequently studied. At first sight its metaphase bivalents appear quite unlike those of all other species, being elongated V-shaped bodies in which the two chromosomes have only just started to separate at the centromeres. The close similarity of these *Callimantis* bivalents to those of the male *Drosophila* and of Muscid flies at once suggested that chiasma formation had been abolished in *Callimantis* as it has in many of the higher Diptera.

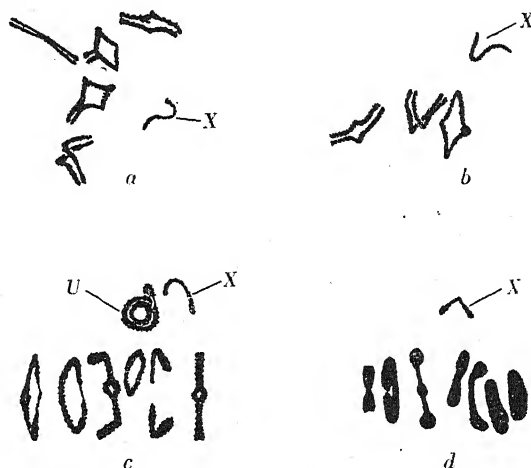
A close examination of abundant material of other species has shown that in most of them (possibly in all) the bivalents go through a *Callimantis*-like stage at the beginning of first metaphase. The only real difference would appear to be that in *Callimantis* the tearing apart process referred to above is postponed until after a relatively long static metaphase stage. The question whether chiasma formation has really been abolished in *Callimantis* is thus reopened: it will be discussed later in § 7 (b).

(g) *Miomantis* sp.

Oöthecae of a *Miomantis* were sent to me by Dr J. Hewitt of Grahamstown, South Africa. Dr H. E. Hinton informs me that it would be unwise to attempt an identification of the species until the taxonomy of the genus has been revised.

This species has 15 chromosomes in the diploid set of the male (Pl. 8, fig. 2). The chromosomes are mediocentric and all about the same size. At meiosis (Pl. 9, fig. 8 and Text-fig. 3a-d) 7 bivalents are formed. The X is negatively heteropycnotic at the first meiotic division. The bivalents at prometaphase have a similar appearance to those of *Acontiothespis*, but owing to the fact that in *Miomantis* the chromosomes are much thinner at this stage the split between the homologous chromosomes can

be seen much more clearly. The details of the tearing apart process can be followed in Text-fig. 3a-d.



Text-fig. 3a-d. *Miomantis*: stages from prometaphase to first metaphase (a and b prometaphases, c early metaphase, d late metaphase). In a and b not all the bivalents are shown, in c and d the complete set are illustrated. In c one bivalent (U) has not undergone the tearing apart process at a time when the others have almost completed it.

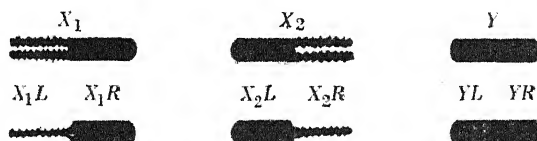
#### 4. THE $X_1X_2Y$ SPECIES

##### (a) *Paratenodera sinensis*

This is a large Asiatic species which has been introduced into the eastern states of the U.S.A. A couple of oöthecae were obtained from a well-known American dealer, and many adult insects were reared in London.

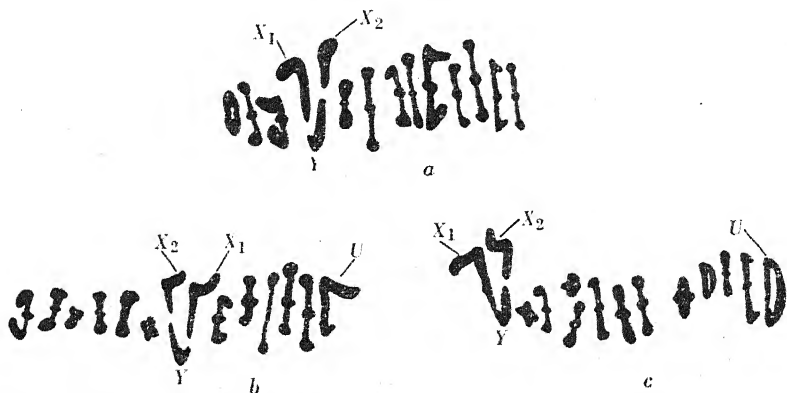
The chromosomes of this species have already been described by King (1931), and there is relatively little that can be added to his account. The diploid number is 27, all the chromosomes being mediocentric. The two X-chromosomes ( $X_1$  and  $X_2$ ) can easily be recognized in the spermatogonial divisions (Pl. 8, fig. 6) because in each chromosome one of the arms is negatively heteropycnotic, while the other is non-heteropycnotic like the autosomes. That these chromosomes with one heteropycnotic arm really are X-chromosomes is shown by their behaviour during the later stages of meiosis. The Y is not distinguishable with any certainty in the spermatogonial metaphases, since its degree of condensation is the same as that of the autosomes.

One of the  $X$ 's is very slightly longer than the other; it will be referred to as  $X_1$ , the shorter one being  $X_2$ . Following the practice of the *Drosophila* workers we may call the two arms of each chromosome right and left ( $R$  and  $L$ ), the designations being quite arbitrary. In the terminology which will be adopted in this paper the two heteropycnotic arms will be referred to as  $X_1L$  and  $X_2R$ , the two non-heteropycnotic arms being  $X_1R$  and  $X_2L$ . The appearance of these arms at a spermatogonial metaphase is shown diagrammatically in Text-fig. 4; in the heteropycnotic arms the chromatids are seen separately in side view, while in the non-heteropycnotic arms the split between them cannot be seen.



Text-fig. 4. *Sphodromantis* spp. and *Paratenodera*: diagrams of the three sex chromosomes as seen in spermatogonial metaphases. Upper row in side view, lower row in polar view. Compare Pl. 8, figs. 5 and 6 and Pl. 9, fig. 7.

At meiosis (Text-fig. 5) the 24 autosomes form 12 bivalents, while the three sex chromosomes form a trivalent in which both the  $X$ 's are paired with the  $Y$ . At first metaphase the three centromeres of the sex trivalent are arranged in the form of an isosceles triangle on the spindle,



Text-fig. 5a-c. *Paratenodera sinensis*: three first metaphases. *a* is from an individual without an unequal bivalent; *b* and *c* are from the same individual with an unequal bivalent ( $U$ ), which may be either  $\Gamma$ -shaped (as in *b*) or ring-shaped (as in *c*).

so that both  $X$ 's pass to one pole at anaphase, the  $Y$  going to the other pole. The exact history of this sex trivalent was not studied so closely



in *Paratenodera* as in some of the other  $X_1X_2Y$  species, so that a description of its origin is best deferred until we come to consider the two species of *Sphodromantis*. It is clear, however, that the two non-heteropycnotic arms of the  $X$ 's ( $X_1R$  and  $X_2L$ ) pair with the two limbs of the  $Y$ , while the other two arms ( $X_1L$  and  $X_2R$ ) are free and unpaired at first metaphase. These two free arms show a certain amount of positive heteropycnosis during the first meiotic division, but it is not so pronounced as in the Orthoptera Saltatoria (White, 1940*a*). Nevertheless, it is clear that a reversal of heteropycnosis occurs in the Mantids, just as it does in the Acrididae and Gryllidae.

If we call the two limbs of the  $Y$   $YR$  and  $YL$ , then the six limbs in the sex trivalent are arranged in the following order:  $X_1L$ ,  $X_1R$ - $YL$ ,  $YR$ - $X_2L$ ,  $X_2R$  (the commas standing for centromeres and the dashes for the points of contact between one chromosome and the next). The relative lengths of all these chromosome arms can be seen clearly in Pl. 10, fig. 17; it will be observed that whereas in  $X_1$  the free limb ( $X_1L$ ) is a good deal shorter than the pairing limb ( $X_1R$ ), in  $X_2$  the two arms are about the same length, the free arm ( $X_2R$ ) being slightly longer than the other. The two free arms are slightly club-shaped at first metaphase; they may stick out laterally, or in almost any other direction, their orientation not being governed by any tension, such as that between the paired arms. The two limbs of the  $Y$  are characteristically unequal in length in *Paratenodera* ( $XL$  being less than half the length of  $XR$ ).

In some first metaphases the pairing arms are still in contact with one another (i.e. the tip of  $X_1R$  touches the end of  $YL$  and the tip of  $X_2L$  touches the end of  $YR$ ); more usually, however, there is a distinct gap between the ends, as in Pl. 10, fig. 17. It would appear that these gaps result from a stretching of the sex trivalent at the time of its orientation on the developing spindle.

The general appearance of the autosomal bivalents at first metaphase is shown in Text-fig. 8. The interpretation of these bivalents will be discussed later (§ 7*b*). Some individuals of *Paratenodera* possess an "unequal" autosomal bivalent (see § 6). It was ascertained that the two halves of this unequal bivalent segregated at first anaphase without regard to the segregation of the sex trivalent.

#### (b) *Tenodera aridifolia*

Oöthecae of this species were sent to me from Kuala Lumpur, Federated Malay States, by Dr N. C. E. Miller. The cytological preparations were not very extensive, and no spermatogonial divisions were

studied. There seems no reason to believe, however, that they differ in any essential respect from those of the last species.

The first metaphase sex trivalent of *Tenodera aridifolia* is shown in Pl. 11, fig. 20. It differs from that of *Paratenodera* in that the two limbs of the Y are approximately equal in length. The proportions of the other four limbs in the sex trivalent are much as in *Paratenodera*.

A number of tetraploid spermatocytes in first metaphase were found in one cyst. Many of the larger autosomes in these cells had formed multivalents, the smaller ones being mostly bivalents, as in other animals with size differences between the chromosomes (White, 1933). Pl. 12, fig. 28 shows one of these tetraploid first metaphases in which there is a "sex quadrivalent" formed by  $X_1-Y-Y-X_1$ . The four centromeres of this configuration are arranged in a zigzag. The two  $X_2$ 's had presumably formed a bivalent elsewhere in the cell, but it was not possible to distinguish it from the many autosomal bivalents.

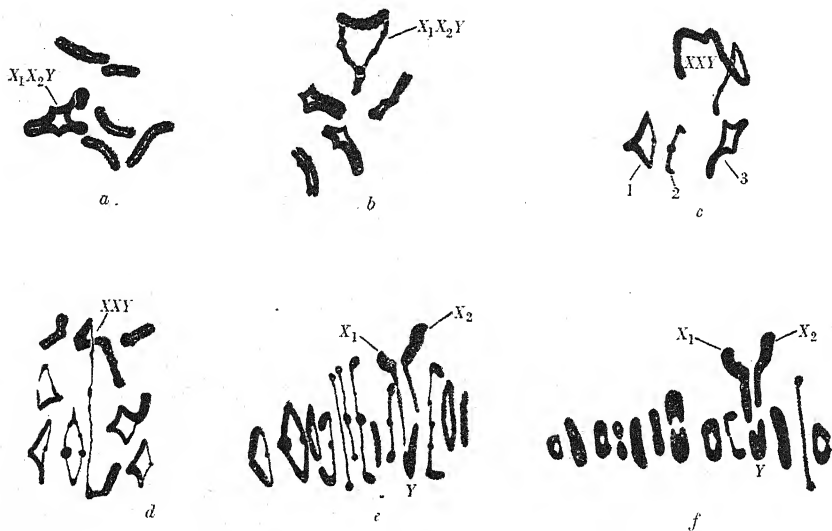
(c) *Sphodromantis* sp.

Several oöthecae of this species were sent to me by Mr E. Bedford of Rustenburg, Transvaal. Owing to the outbreak of war it was unfortunately not possible to rear adults, and the systematic determination of the material must hence remain uncertain, although Dr B. P. Uvarov informs me that there can be no doubt that the nymphs belong to a member of the genus *Sphodromantis*. The cytological preparations of this species were very fine and extensive, and the material was studied very thoroughly.

The spermatogonial metaphases do not differ in any important respect from those of *Paratenodera*, and the chromosome number was the same (27). Various stages of meiosis are shown in Pls. 10-13, figs. 15, 19, 22, 25, 32, 34 and 35 and in Text-figs. 5 and 6.

At pachytene a large heteropycnotic mass can be seen at one side of each nucleus. To this mass is attached an apparently continuous ring from which project two lateral branches. A study of later stages makes it clear that this structure represents the sex trivalent. A similar structure was found in *Paratenodera*, and is illustrated in Pl. 13, fig. 31. It would appear that the pachytene sex trivalent should be interpreted thus (Text-fig. 12): the heteropycnotic mass represents  $X_1L$  and  $X_2R$  which have fused together to form a body whose exact structure cannot be made out since it is "unfixable" (see White, 1940a). The other two projections from the ring are the end of  $X_1R$  paired with  $YL$  and that of  $X_2L$  paired with  $YR$ .

The more important of the later stages of meiosis are illustrated in Text-fig. 6*a-f*. In *a*, which is taken from a cell in prometaphase, the autosomal bivalents are slightly curved rods down the middle of which an indistinct split is visible. No trace of chiasmata can be seen at this stage, and it would seem that the two chromosomes of each bivalent are still paired throughout their length. The sex trivalent is a ring from which three lateral branches project, the branch which is composed of  $X_1L$  and  $X_2R$  having lost its conspicuous heteropycnosis since pachytene.

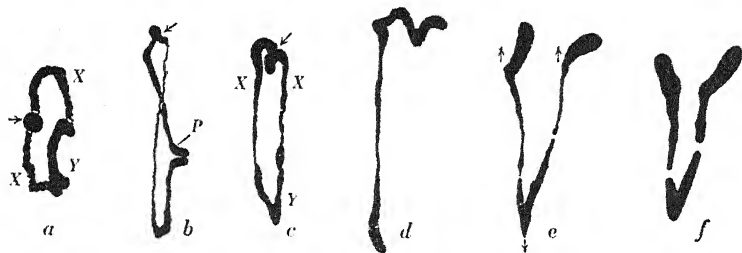


Text-fig. 6*a-f*. *Sphodromantis* sp.: stages from prometaphase to first metaphase (*a-d* prometaphases, *e* early metaphase, *f* late metaphase). In *a-d* only a few of the bivalents are shown, in *e* and *f* the whole set are illustrated. In *a* the tearing apart process has not yet begun, in *b-d* it is proceeding, while in *e* and *f* it is completed. The temporary prophase association between  $X_1L$  and  $X_2R$  is seen in *a* and *b* but no longer exists in later stages. The sex trivalent is incompletely orientated on the spindle in *d*; in *e* and *f* its orientation has been completed.

At a slightly later stage of prometaphase, when the spindle is beginning to form (Text-fig. 6*b*), the centromeres of the autosomal bivalents begin to separate, so that a diamond-shaped space makes its appearance between the chromosomes of each bivalent. At about the same time the sex trivalent ceases to be a ring, through a separation of  $X_1L$  and  $X_2R$ , which become free. This separation does not seem to be due to any active tearing apart, since these arms often separate before the spindle begins to form, and they are never drawn out into thin threads in a manner which would suggest tension. More probably their separation is analogous to that which occurs in many of the *XO* species of Orthoptera where the

X-chromosomes are bent double in pachytene with the two limbs in close contact, straightening out later, before metaphase.

Very soon after the sex trivalent has become an open chain all the chromosomes appear to be violently stretched on the developing spindle, the degree of stretching being indicated by the way in which the chromosomes become drawn out into thin threads (Text-fig. 6*d, e*). Very often there is a lack of synchronization in the cell at this stage, so that some of the bivalents may be stretched and drawn out, while others are still in a stage corresponding to early prometaphase. The orientation of the sex trivalent on the spindle is frequently incomplete at the "stretching stage", so that one of the X's and the Y are toward one pole, the second X toward the other (Text-figs. 6*d, 7d*). This mal-orientation is usually a



Text-fig. 7*a-f*. *Sphodromantis* sp.: various stages in the development of the sex trivalent from prometaphase to first metaphase. In *a-c* the non-permanent association between  $X_1L$  and  $X_2R$  causes the sex trivalent to appear as a continuous ring; in the later stages this association has come to an end.

transient phase which precedes the correct orientation of the metaphase stage proper; but occasionally it persists, as we shall see later.

The diamond-shaped spaces in the autosomal bivalents have gradually got larger and larger during prometaphase and early metaphase. When the violent stretching occurs some of the bivalents remain as rings, while in others the connexion between one pair of arms breaks, so that a "rod bivalent" is left. The number of rod bivalents in *Sphodromantis* sp. is usually 2-6, the remaining 6-10 being ring bivalents. This is a point of difference from *Paratenodera*, where nearly all the autosomes form rod bivalents (see Text-fig. 5). Two stages in the transformation of prometaphase bivalents into metaphase ones are shown in Pl. 13, figs. 34 and 35. In these and in one of the bivalents on the right-hand side of Text-fig. 6*d*, an interesting point of doubtful significance can be observed. Frequently one side of the diamond-shaped area is much thinner than the one opposite and homologous to it. It would seem that the nucleic acid is in some way shared by the two chromosomes at

prometaphase, and that when the tearing apart process occurs it may be distributed unequally, so that a particular region in one chromosome receives more nucleic acid than the homologous region in the other chromosome.

A comparison between Text-fig. 6e with f will show the difference between early and late metaphase in this species. In the late metaphase the chromosomes have shortened and thickened considerably. There can be no doubt about the exact sequence of the stages, since the late metaphases are found in the same cyst as anaphases and second division cells, while the early metaphases are found next to prometaphases and earlier stages. In the late metaphases the ends of the chromosomes in the autosomal bivalents are still in contact, but in the sex trivalent there are definite gaps between the ends of the "pairing arms".

An idea of the relative lengths of the chromosome arms in the sex trivalent can be obtained from Text-fig. 6e, f and also from Pl. 11, fig. 19. The proportions of  $X_1$  and  $X_2$  are very much as in *Paratenodera*,  $X_1R$  being the longest of the six limbs in the trivalent. The two limbs of the Y are unequal in length,  $YL$  being distinctly shorter than  $YR$ , although the difference in length is not so great as in *Paratenodera*.

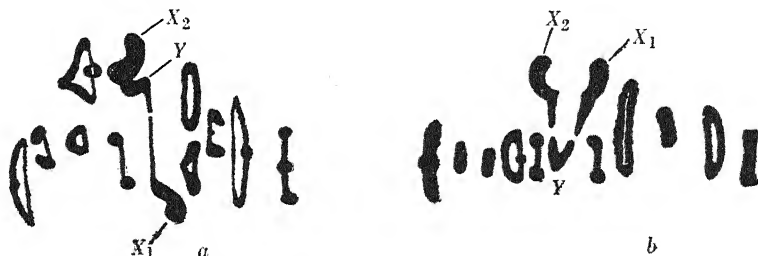
(d) *Sphodromantis viridis*

Numerous oöthecae of this species were obtained from Algeria through the courtesy of Dr A. Cros. It was formerly known as *Sphodromantis bioculata*.

Since all the other  $X_1X_2Y$  species have 27 chromosomes in the male, it was a surprise to find that this one only possessed 23. All these chromosomes were mediocentric, as in the other  $X_1X_2Y$  species. A spermatogonial metaphase of *S. viridis* is shown in Pl. 8, fig. 5. The two X-chromosomes can be recognized by the negative heteropycnosis of one limb in each of them. The Y is not distinguishable from the autosomes in the spermatogonia.

At meiosis this species forms ten autosomal bivalents and a sex trivalent which closely resembles that of the other  $X_1X_2Y$  species (Pl. 10, figs. 14, 17). A very early and a later stage of the first metaphase are drawn in Text-fig. 8a, b. The general course of meiosis in this species is very much as in the last one, but the tearing apart process seems to be less violent, since the chromosomes are not drawn out into such long thin threads as they are in the South African species. *S. viridis* usually seems to show about three rod bivalents, the remainder being rings. The three chromosomes in the sex trivalent resemble those of the

former species, except that *YL* and *YR* are almost exactly the same length, as in *Tenodera aridifolia*.



Text-fig. 8a, b. *Sphodromantis viridis*: a very early and a late first metaphase. In a the orientation of the sex trivalent on the spindle has not been completed; in b the sex trivalent has become properly orientated.

##### 5. NON-DISJUNCTION OF THE SEX CHROMOSOMES IN THE $X_1X_2Y$ GENERA

In three of the  $X_1X_2Y$  species (*Paratenodera* and the two species of *Sphodromantis*) non-disjunction of the sex chromosomes takes place fairly frequently at the first meiotic division. It probably also occurs in *Tenodera aridifolia*, but my material of that species was very limited. Non-disjunction leads to the production of gametes which instead of carrying  $X_1+X_2$  or  $Y$  carry  $X_1+Y$ ,  $X_2+Y$ ,  $X_1$  or  $X_2$ . The fate of the gametes bearing these abnormal combinations of sex chromosomes is not known with certainty; there is no evidence that they perish before leaving the testis, nor is there any evidence that  $X_1X_1X_2Y$ ,  $X_1X_2X_2Y$ ,  $X_1X_1X_2$  or  $X_1X_2X_2$  adults are ever produced. In *Drosophila* it is known that sperms carrying abnormal chromosome sets are capable of penetrating the egg, although most of the zygotic combinations they give rise to on fertilization are non-viable. It is highly probable that the same occurs in the  $X_1X_2Y$  Mantids, but no definite evidence exists.

The non-disjunction observed in *Paratenodera* and the two species of *Sphodromantis* appears to result from two different causes. In the first place a small percentage of the sex trivalents fail to become oriented properly on the spindle at metaphase. It has been pointed out that this mal-orientation is frequent during early metaphase—usually as metaphase proceeds the orientation of the sex trivalent corrects itself, but occasionally the mal-orientation seems to persist right up to anaphase. In some cases the three centromeres of the sex trivalent become arranged almost in a straight line so that the  $Y$  is sketched in the middle of the spindle between the two  $X$ 's; this type of mal-orientation is shown in

Pl. 11, fig. 21. In other cases the  $Y$  and one of the  $X$ 's become orientated towards one pole, the other  $X$  towards the opposite pole (Pl. 11, fig. 22). There is no sharp distinction between these two types of mal-orientation, since intermediates frequently occur.

The second cause of non-disjunction is that sometimes one of the  $X$ 's fails to pair with the  $Y$ . In this case instead of a sex trivalent we have a sex bivalent and a univalent. In this case the univalent may pass to either pole at anaphase. It will be seen from Table 1 that failure

Table 1

Cyst no.	Normal	Mal- orientated	Failure of pairing			Total
			$\widehat{X_1 Y} + X_2$	$X_2 \widehat{Y} + X_1$	Undetermined	
<i>Paratenodera</i>						
1	127	—	3	1	—	
2	145	—	4	—	1	
3	103	—	3	1	—	
4	97	6	1	1	1	
5	37	—	—	—	—	
6	26	—	—	—	—	
	535	6	11	3	2	557
<i>Sphodromantis</i> sp.						
1	159	10	11	2	6	
2	186	3	3	—	3	
3	132	4	6	1	1	
4	126	—	—	1	1	
5	73	8	—	—	—	
6	—	—	4	—	5	
7	8	—	—	—	—	
	684	25	24	4	16	753
<i>Sphodromantis viridis</i>						
1	148	—	—	—	4	
2	119	—	—	—	9	
3	149	4	—	—	2	
4	53	1	—	—	—	
5	12	4	—	—	—	
	481	9	—	—	15	505

of pairing is distinctly commoner in *Sphodromantis* sp. than in either of the two other species. Since either  $X_1$  or  $X_2$  may fail to pair with the  $Y$  there are two types of failure of pairing:  $\widehat{X_1 Y} + X_2$  and  $X_2 \widehat{Y} + X_1$ . In *S. viridis* it is not possible to distinguish these two types with certainty, since  $X_1$  and  $X_2$  are very much alike and the two arms of the  $Y$  are almost equal in length. In the other two species, however, the two types of non-pairing can usually be distinguished. In some cases however it is difficult to classify a cell showing failure of pairing into one or other of the two types; this is usually the case when the sex bivalent lies vertically in the slide. It will be seen from Table 1 that both in *Paratenodera*

and *Sphodromantis* sp. the  $\widehat{X_1Y} + X_2$  condition is commoner than the  $\widehat{X_2Y} + X_1$  one. The first condition is shown on Pl. 11, figs. 23, 24 and Pl. 12, fig. 27 (taken from *S. viridis*, *Sphodromantis* sp. and *Paratenodera*), and the second condition is illustrated on Pl. 12, figs. 25, 26 (taken from *Sphodromantis* sp. and *Paratenodera*).

It is not clear just how failure of pairing arises. There are three theoretical possibilities: (1) that one  $X$  is separate from zygotene right up to metaphase, (2) that the  $\widehat{X_1Y} + X_2$  and  $\widehat{X_2Y} + X_1$  conditions arise from ring trivalents such as that illustrated in Pl. 13, fig. 31, one  $X$  becoming detached from the  $Y$  just before metaphase, at the time when  $X_1L$  becomes free from  $X_2R$ , (3) the detachment of one of the  $X$ 's from the  $Y$  may take place even after the formation of the spindle, i.e. at first metaphase. The fact that no univalent  $X$ 's have been seen during prophase or prometaphase is evidence against the first hypothesis and in favour of either the second or the third. There is also some evidence that the proportion of cells showing failure of pairing is greater in late metaphases than in early ones. Thus in *Sphodromantis* sp. a number of cysts were observed in which the majority of the cells were in interkinesis or second metaphase. Often these cysts contained a few cells which had remained "blocked" in first metaphase; it was observed that such cells nearly always showed failure of pairing of either the  $\widehat{X_1Y} + X_2$  or the  $\widehat{X_2Y} + X_1$  type. Cyst no. 6 in *Sphodromantis* sp. (see Table 1) was a cyst of this kind. The existence of these "blocked" cells with failure of pairing would seem to suggest that one of the  $X$ 's may break away from the sex trivalent at metaphase, perhaps through excessive stretching of the trivalent on the spindle.

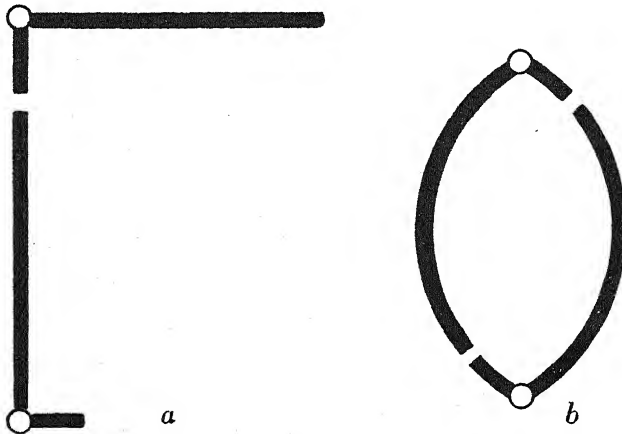
The total amount of non-disjunction in the three species can be worked out from Table 1 if one assumes that every mal-orientation gives rise to non-disjunctional sperms, whereas failure of pairing does so in 50% of all cases. On these assumptions the amount of non-disjunction in the material studied was 2.51% in *Paratenodera*, 6.24% in *Sphodromantis* sp., and 3.27% in *Sphodromantis viridis*.

#### 6. UNEQUAL BIVALENTS OF *TENODERA* AND *PARATENODERA*

In one individual of *Tenodera* (out of a total of two) and in one of *Paratenodera* (out of a total of four) unequal bivalents were found (Pl. 12, figs. 29, 30). In both cases only one bivalent in the nucleus was unequal. At first sight these unequal bivalents appear to have the



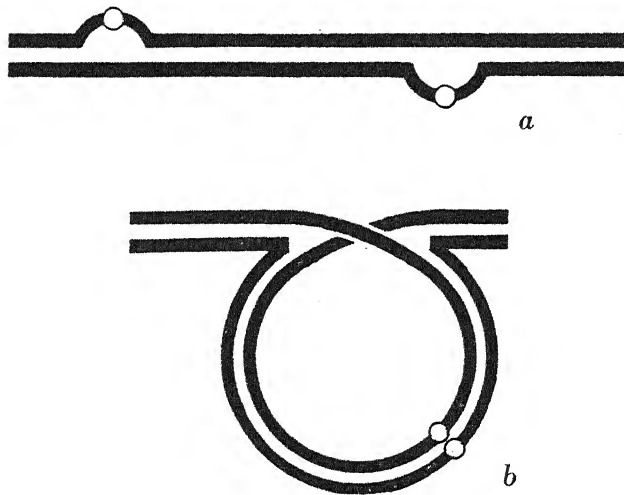
shape  $\Gamma$ , but a more detailed examination shows that they have the shape represented diagrammatically in Text-fig. 9 a. Both the chromosomes of which the bivalent is composed are mediocentric, but one arm is in each case much longer than the other: the long arm of one chromosome is paired with the short arm of the other. Although it is difficult to be absolutely certain, both chromosomes appear to be the same length in *Tenodera*, while in *Paratenodera* one is somewhat longer than the other (cf. Pl. 12, figs. 29, 30). In the individual of *Paratenodera* carrying the unequal bivalent a few first metaphases were found in which no  $\Gamma$ -shaped bivalents could be seen; but these metaphases always possessed a rather large ring bivalent whose structure can probably be represented



Text-fig. 9 a, b. Diagrams of the two types of unequal bivalents shown in Text-fig. 5 b, c.

diagrammatically in Text-fig. 9 b. This shows that the terminal regions of the two long arms are homologous to those of the two short ones, although the individual was heterozygous for some sort of structural rearrangement in the middle part of this chromosome. It seems probable that this rearrangement was either a centric shift (translocation of a small region containing the centromere to another position in the same chromosome) or a pericentric inversion (inversion of a region containing the centromere). At present it seems impossible to decide between these two alternatives. Both types of rearrangement have been previously recorded from wild populations, but only very rarely. The unequal bivalents of *Tenodera* and *Paratenodera* are clearly different in nature from those recorded by McClung (1928), Wenrich (1916), Carothers (1931) and Darlington (1936) in the grasshoppers *Mecostethus*, *Phrynotettix*

*Amphitornus* and *Stauroderus*. In these cases the position of the centromere is the same in both homologues, but one of the latter is longer than the other, due to deficiency or duplication. The Mantid unequal bivalents are more nearly analogous to those described by Klingstedt (1933) in the neuropteran *Hemerobius*, and to those studied by Carothers (1917), King (1923) and Helwig (1929) in the grasshopper genera *Circotettix* and *Trimerotropis*. In some species of these two genera many unequal bivalents may occur in the same individual, the two chromosomes being of about the same length, although one is telocentric and the other mediocentric. But the unequal



Text-fig. 10a, b. Diagrams of pachytene pairing in a bivalent heterozygous for a small pericentric shift and a large pericentric inversion.

bivalents of *Circotettix* and *Trimerotropis* do not seem ever to form ring bivalents as those of *Paratenodera* sometimes do.

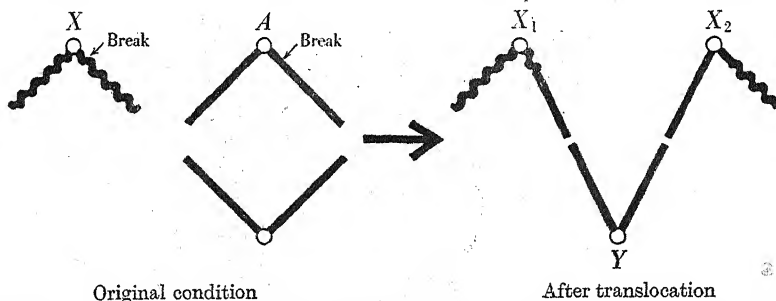
*Paratenodera sinensis* and *Tenodera aridifolia* are taxonomically very close together and have been included in the same genus by many authorities. It is therefore possible that the structural rearrangement responsible for the appearance of the unequal bivalent in the two species is the same one, having been inherited from their common ancestor. This, however, is purely speculative; a rearrangement of similar type may have occurred independently in the two species. There is no evidence that the chromosome involved is the same one in the two species, although it is of similar size in both.

## 7. DISCUSSION

## (a) The evolution of the sex-chromosome mechanism in Mantids

Most Mantids probably possess a single  $X$ -chromosome in the male as in *Iris*, *Ameles*, *Callimantis*, *Acontiothespis* and *Miomantis*. This situation is also found in the cockroaches (which are the group of insects most nearly related to the Mantids), so that it is probably one which is of very great antiquity. Usually the  $X$  is one of the medium-sized chromosomes, but in the Empusinae—which are a highly specialized group—the  $X$  has become relatively enormous, so that in *Gongylus* it is by far the largest chromosome in the set. Such an extraordinary enlargement of the  $X$ -chromosome is unparalleled elsewhere, except in some of the long-horned grasshoppers of the subfamily Phasgonurinae (Asana *et al.* 1938; White, unpublished).

In all the  $XO$  Mantids investigated at present the  $X$  is a medio-centric chromosome whose arms are of approximately equal length. This



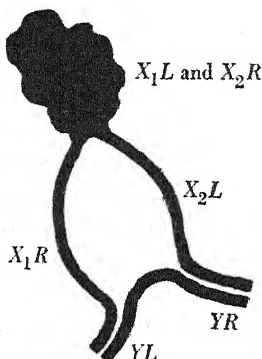
Text-fig. 11. Diagram showing the presumed origin of the  $X_1X_2Y$  mechanism from the  $XO$  one.  $A$  = a pair of autosomes.

situation contrasts with that found in the long-horned grasshoppers, where both telocentric and medio-centric  $X$ -chromosomes are found, even in the same subfamily.

Since there is no evidence that any Mantid has an  $XY$  mechanism it appears probable that the  $X_1X_2Y$  system arose from the simple  $XO$  condition. In a previous paper (White, 1940*b*) it has been suggested that this transformation took place as a result of a translocation between the  $X$  and a medio-centric autosome (Text-fig. 11). The translocation must have been a "mutual" one involving two breakage points, each very close to the centromere of its own chromosome; it resulted in an exchange of two chromosome arms and gave rise to a new type of sex-chromosome mechanism without altering the "genic balance" in either sex. The  $Y$  in the new mechanism was originally an autosome and has

only become confined to the male line since the translocation became established. It is consequently a "neo-Y" analogous to those which have arisen in the grasshoppers *Mermiria*, *Hesperotettix* and *Paratytopidia* (McClung, 1917; King & Beams, 1938; White, 1940*b*).

This theory of the origin of the  $X_1X_2Y$  mechanism is strongly supported by a study of the existing species. The chromosome arms which we have designated  $X_1L$  and  $X_2R$  must be regarded as the two limbs of the original  $X$ ; this view is supported by the following facts: (1) they are negatively heteropycnotic in the spermatogonial metaphases, (2) they stick together in a special manner during the prophase of the first meiotic division without undergoing any true pairing, (3) they are always free and unpaired at first metaphase, thus showing that they



Text-fig. 12. Diagram showing the structure of the sex trivalent at pachytene. Cf. Pl. 13, fig. 31.

are not homologous to one another or to any other chromosome in the set.

If  $X_1L$  and  $X_2R$  represent the original  $X$ , then the four remaining chromosome arms of the sex trivalent ( $X_1R$ ,  $YL$ ,  $YR$ ,  $X_2L$ ) must have originally constituted a pair of mediocentric autosomes. One member of this pair gave rise to  $X_1R$  and  $X_2L$ . Either the centromere of  $X_1$  or that of  $X_2$  represents that of the original  $X$ , while the other is autosomal in origin—but it does not appear possible to decide which is which.

It will be realized that on this hypothesis  $X_1R$  should be homologous to  $YL$  and  $X_2L$  to  $YR$ . In the modern species this homology appears to be restricted to the distal ends of the chromosome arms. Thus each arm consists of a distal pairing segment and a proximal differential one. Such a condition is almost certainly a secondary one which has arisen in the course of evolution since the origin of the  $X_1X_2Y$  system. It may be

the result of a number of small structural rearrangements (inversions, etc.) in the proximal parts of  $X_1R$ ,  $YL$ ,  $YR$  and  $X_2L$ .

Of the three chromosomes in the sex trivalent the  $Y$  shows by far the greatest amount of interspecific variation. In *Stagmomantis carolina* (King, 1931) and in *Hierodula* sp. (Asana, 1934) it is apparently quite small, while in *Tenodera*, *Paratenodera*, *Mantis* and *Sphodromantis* it is very much larger. In *Tenodera aridifolia* and *Sphodromantis viridis* its two limbs are practically equal in length, while in *Paratenodera* and *Sphodromantis* sp.  $YL$  is much shorter than  $YR$ . It is impossible to determine which of these conditions (if any) was the original one; it is, however, clear that since the origin of the  $X_1X_2Y$  mechanism a number of structural rearrangements have taken place in the neo- $Y$ , altering its shape in various ways. These changes probably began with the building up of a differential region round the centromere (by successive inversions). Once such a differential region had arisen it is not surprising that it underwent a large number of other structural changes, since a region which is present in one sex only and does not undergo crossing-over is a particularly favourable situation for structural changes to become established.

If genetically inert regions are always heteropycnotic the  $Y$  in the  $X_1X_2Y$  Mantids (which is not heteropycnotic) must be genetically "active". Since it contains a differential segment which cannot cross-over with either of the  $X$ 's there should be a number of  $Y$ -linked genes in these  $X_1X_2Y$  Mantids. A genetical study of a species with a "neo- $Y$ " which has arisen from an autosome in the not very distant past would be of considerable evolutionary interest.

We have assumed that the  $X_1X_2Y$  mechanism arose in a single species from which all the present-day  $X_1X_2Y$  genera have been derived.<sup>1</sup> The evidence for this view is of two kinds: in the first place it has been shown that  $X_1$  and  $X_2$  can be distinguished from one another and homologized in at least four species.  $X_1$  always has a short free arm ( $X_1L$ ) and a long pairing arm ( $X_1R$ ), while the length relationship is the reverse in  $X_2$  (the pairing arm being a little shorter than the free arm).

<sup>1</sup> Muller (1940, p. 217) has pointed out that whereas gene mutations may recur with a frequency of the order of one in a million gametes, each structural rearrangement constitutes a virtually unique case. This is because it depends on at least two independent breaks. A given structural rearrangement will consequently recur with a frequency which is the product of the frequencies of the breaks involved. The exact position of the breaks along the length of the chromosome can, of course, only be determined where salivary chromosomes are present or where the breakage points have been localized by genetical methods. In the present instance the breakage points could only be determined very approximately by comparing the lengths of  $X_1R$  and  $X_2L$  in the four species studied.

Such a constant relation between these four chromosome arms in all the species studied can scarcely be due to chance. In the second place the six  $X_1X_2Y$  genera are systematically close to one another, so that a common origin is highly plausible on taxonomic grounds alone. The total number of species included in these genera is 110 according to Giglio-Tos (1927), so that the  $X_1X_2Y$  system must have originated quite a long time ago—say in early tertiary times. Other closely related genera which may be expected to have an  $X_1X_2Y$  mechanism are *Polyspilota* and the numerous genera created by Giglio-Tos and included by him in his groups Stagmomantes, Polyspilotae, Mantes, Tenoderæ and Hierodulæ. These five groups may be considered to form a natural unit (Mantinae *sensu stricto*) whose relationship to the other genera (e.g. *Callimantis*, *Miomantis*) traditionally included in the Mantinae is probably not very close. The Mantinae (*sensu stricto*) include 191 species according to Giglio-Tos's monograph (and there are doubtless many undescribed species); it is not often that one can determine with the degree of certainty which exists in the present case that such a large assemblage of genera and species have been derived from a single ancestral species. The  $X_1X_2Y$  Mantids are mainly an Old World group, but the genus *Stagmomantis* is found in both North and South America.

None of the  $XO$  species which have been studied in the present investigation appears to be closely related to the hypothetical form in which the  $X_1X_2Y$  system first arose. Such a hypothetical ancestor probably had a chromosome number which was 27 (in the male), or at any rate not very far from that number; it may be presumed to have had a mediocentric  $X$ -chromosome of moderate length which was negatively heteropycnotic in the spermatogonial metaphases as  $X_1L$  and  $X_2R$  are in the present-day  $X_1X_2Y$  forms. None of the  $XO$  species described in this paper fulfils the last requirement—their  $X$ 's are not distinguishable by any heteropycnosis at the spermatogonial metaphases.

No mention has been made in the above discussion of the work of Williams (1938) on the Mantid *Choeradodis rhombicollis*. This work was carried out on an animal which appears to have been fixed entire several years before. The author claims that this species has 27 chromosomes and an  $X_1X_2Y$  trivalent like that of all the Mantids investigated before 1938. If this account is trustworthy the Asiatic and Central American genus *Choeradodis* must be regarded (in spite of its specialized pronotum) as a member of the Mantinae *sensu stricto*, since it can hardly have acquired an  $X_1X_2Y$  mechanism of the same type independently. This

involves the disappearance of the subfamily Choeradodinae hitherto recognized by taxonomists.

With the single exception of *Sphodromantis viridis* all the  $X_1X_2Y$  species which have been studied by Oguma, King, Asana and myself have the same chromosome number (27 in the male). *S. viridis* has probably lost two chromosome pairs since the origin of the  $X_1X_2Y$  system. It is, of course, not necessary to suppose that all the genetic material in those chromosomes was actually lost—probably it was merely transferred to other chromosomes by a series of translocations, the regions containing the centromeres which were actually lost being very small.

The Mantids seem to show about as much variation in chromosome number as the Gryllidae and Tettigonidae. These three groups all show a far greater range of variation in this respect than the Acrididae, which are morphologically a more uniform group and possibly a more recent one.

Nothing definite is known about the ancestry of the group Mantoidea. There seems to be little doubt that it is to be regarded as a specialized offshoot from the Blattoid stock, but there is no palaeontological evidence as to when it first arose. Certain liassic fossil insects which were formerly believed (by Handlirsch) to be Mantids have recently been shown to belong to the Prophalangopsidae, a group of the Ensifera (Zeuner, 1939).

#### (b) *The meiotic mechanism in Mantids*

In a previous paper (White, 1938) it was pointed out that the meiosis of *Callimantis* differs from that of other animals in some essential respects. At that time I had not had an opportunity to examine the chromosomes of any other Mantid species. The ten other species subsequently investigated have a meiotic mechanism which in general seems to resemble that of *Callimantis*, although there are some important differences of detail. In none of the species can true chiasmata be seen, since the stages of diplotene and diakinesis are highly modified. It is quite possible that crossing-over may occur at pachytene; but if so it does not give rise to any visible exchanges of chromatids. The chromosomes which have paired at zygotene remain closely approximated along their entire length until the beginning of metaphase. At this stage the spindle begins to form and the centromeres of the bivalents become co-orientated above and below what will be the equatorial plane of the completed spindle. As this is happening the centromeres of each bivalent begin to separate from each other, thereby forcefully tearing apart the

two chromosomes of which the bivalent is composed. In *Callimantis* this process of tearing apart is interrupted very soon after it has begun, to be resumed later, at anaphase. Thus in this genus the metaphase bivalents are V-shaped structures whose arms lie in the "horizontal" plane: they have a strong superficial resemblance to mitotic chromosomes and a real similarity to the autosomal bivalents of Muscid flies (Stevens, 1908), where we have every reason to believe that crossing-over does not occur in the males (Darlington, 1934).

In the other species of Mantids studied the tearing apart takes place at prometaphase. It is not, however, quite completed at this stage, since the ends of the chromosomes (or at any rate some of them) remain in contact during metaphase. Those bivalents in which both ends remain in contact may be called "ring bivalents", those in which only one end remains in contact "rod bivalents". The relative abundance of these two types seems to be characteristic of each species; thus in *Paratenodera* (Pl. 9, fig. 8) nearly all the chromosomes form rod-bivalents, while in the two species of *Sphodromantis* (Pl. 8, fig. 5 and Pl. 9, fig. 7) at least three or four bivalents are usually ring-shaped.

As already stated it cannot be decided on purely cytological grounds whether crossing-over occurs in male Mantids. The evidence against its occurrence consists in the absence of visible chiasmata at all stages of meiosis. In *Callimantis* especially, the similarity of the meiotic bivalents to those of *Drosophila* and *Calliphora* was regarded as conclusive evidence for the absence of chiasma formation and crossing-over (White, 1938). The evidence in favour of the existence of crossing-over is of an indirect and rather unsatisfactory kind, but it would be wrong to disregard it entirely. It is of two kinds. In the first place occasional metaphase bivalents have been seen in *Miomantis* and in *Sphodromantis viridis* in which a separation of pairs of chromatids had taken place at one end of the bivalent before the tearing apart was completed. This gives rise to the appearance seen in Text-fig. 2c and Pl. 13, fig. 36. Such appearances might be interpreted as chiasmata, but they are so rare that it is doubtful if they should be regarded as significant.

In the second place any interpretation of meiosis should explain why in species such as *Miomantis*, *Sphodromantis* spp. and *Paratenodera* some of the chromosomes form ring bivalents while others form rod bivalents. It might reasonably be suggested that ring bivalents have a cross-over in both arms, while rod bivalents have one in one arm only. If this interpretation is correct the difference between the meiosis of the Mantoidea and that of other organisms is a purely superficial one;



crossing-over occurs at pachytene, but cross-overs do not become visible as chiasmata owing to the fact that all four chromatids of which the bivalent is composed remain closely paired until metaphase.

Whichever interpretation is adopted it seems clear that the differences between *Callimantis* and the other Mantids are superficial and do not indicate that crossing-over is present in some Mantids, but absent in others. The mechanism of meiosis is probably fundamentally the same in all Mantids, and a similar mechanism is probably also found in the Blattoidea—to judge from the figures published by Morse (1909) for *Periplaneta*. There is nothing surprising in this, since the Blattoidea and the Mantoidea are generally regarded as closely related.

The history of the sex trivalent in the  $X_1X_2Y$  species suggests that the meiotic mechanism in the paired regions is essentially the same as in the autosomes. The tearing apart process begins at the same time, but it seems to be rather more violent than in the autosomal bivalents, since it usually leads to a complete separation of the homologous chromosome ends, between which clear gaps can be seen at metaphase (Pl. 10, figs. 17, 18). Similar gaps are also seen where one of the  $X$ 's is unpaired, so that there is a sex bivalent. On any theory which assumes that an active force of repulsion is responsible for the tearing apart of the bivalents this force should vary inversely as the square of the distance between the centromeres. Now when the tearing apart process begins the three centromeres of the sex trivalent are definitely farther apart than the two which are present in an ordinary autosomal bivalent. If an active repulsion were responsible for the tearing apart, we should consequently expect that the latter process would be less violent in the sex trivalent than in the autosomal bivalents. If, on the other hand, the tearing apart process is due to a sudden growth of the spindle, to which the centromeres are immovably attached, then we might expect that the gaps between the ends of the sex chromosomes would appear earlier than those between the autosomes, since the paired regions in the sex trivalent must be very short—much shorter than any of the autosomal arms which are paired throughout their whole length.

## 8. SUMMARY

1. The majority of the Mantoidea have an  $XO : XX$  sex-determining mechanism like that found in the other Orthopteroid groups. The  $X$  seems to be without exception a mediocentric chromosome.

2. In one group of genera (subfamily Mantinae *sensu stricto*) an  $X_1X_2Y : X_1X_1X_2X_2$  mechanism has been developed. It may reasonably

be assumed that this mechanism arose in the first place by a mutual translocation as a result of which a pair of autosomes became involved in the sex-determining mechanism.

3. The diploid chromosome numbers of the Mantids vary from 15 to 29 in the fifteen species which have been investigated. All the  $X_1X_2Y$  species studied (with the exception of *Sphodromantis viridis*) have 27 chromosomes. *S. viridis* has only 23.

4. In all the species studied meiosis seems to be of an anomalous type which differs in important respects from the process found in the Orthoptera Saltatoria. It seems impossible to determine by purely cytological means whether crossing-over occurs, since chiasmata are not seen.

I am particularly indebted to a number of entomologists in the tropics who went to very considerable trouble in order to send me material for this investigation. Among those I should particularly like to thank are the following: Messrs N. C. E. Miller (Federated Malay States), G. M. Henry (Ceylon), Dr A. Cros (Algeria), M. M. Legge (Sudan), E. Bedford (South Africa), Prof. M. A. Moghe (Central Provinces, India), Dr Carlos A. Marelli (La Plata), J. Hewitt (South Africa), E. B. Edney (Southern Rhodesia), M. E. Walsh (Java), F. L. Vanderplank (Tanganyika), Dr Lieftinck (Java), B. Peers (Capetown).

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To Dr C. D. Darlington I am indebted for allowing me to complete this investigation at the John Innes Horticultural Institution after the outbreak of war.

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## EXPLANATION OF PLATES 8-13

## PLATE 8

- Fig. 1. *Acontiothespis* sp. Spermatogonial metaphase. Fifteen mediocentric chromosomes.
- Fig. 2. *Miomantis* sp. Spermatogonial metaphase. Fifteen mediocentric chromosomes.
- Fig. 3. *Empusa egena*. Spermatogonial metaphase. Twenty-seven mediocentric chromosomes, the X being the longest.
- Fig. 4. *Gongylus gongyloides*. Spermatogonial metaphase. Twenty-seven mediocentric chromosomes, the X being the longest.

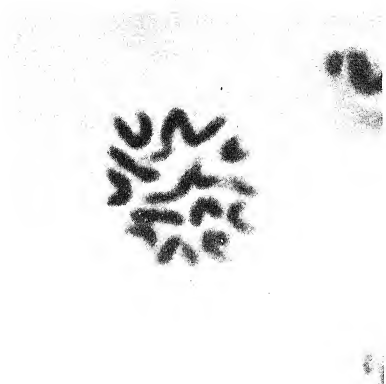


Fig. 1.



Fig. 2.

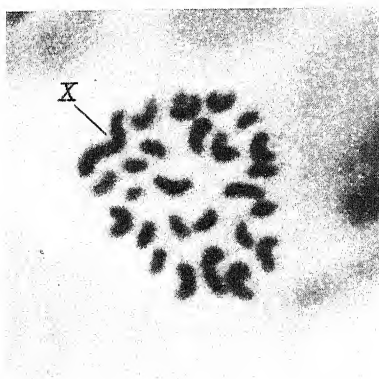


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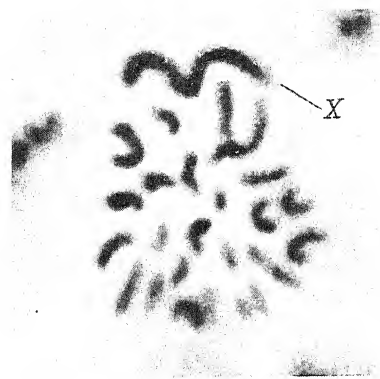


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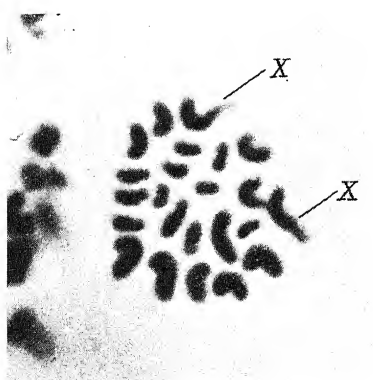


Fig. 5.

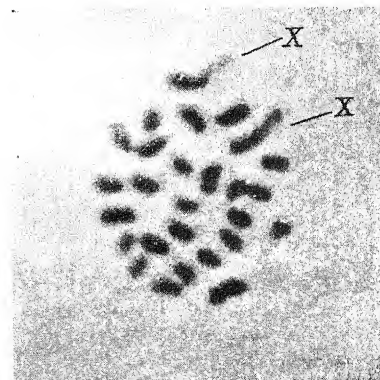


Fig. 6.



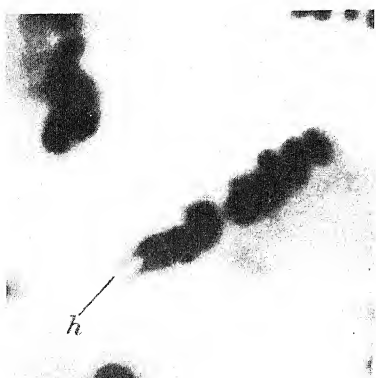


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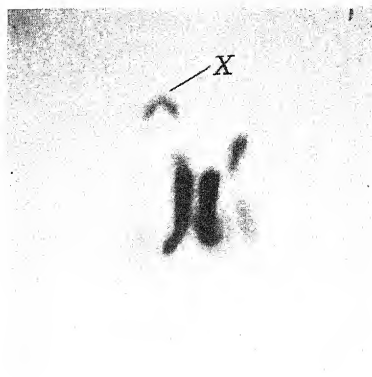


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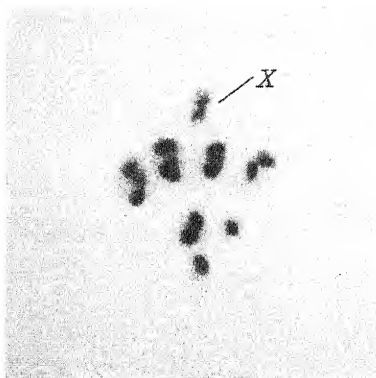


Fig. 9.



Fig. 10.

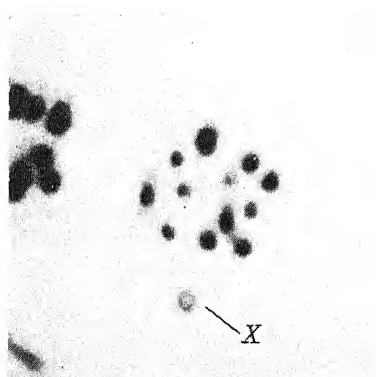


Fig. 11.

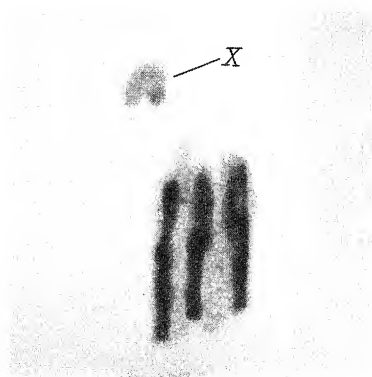


Fig. 12.





Fig. 13.

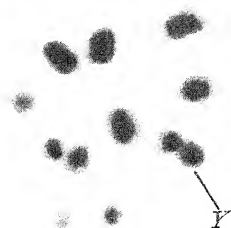


Fig. 14.

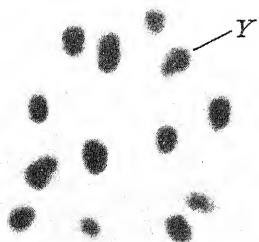


Fig. 15.

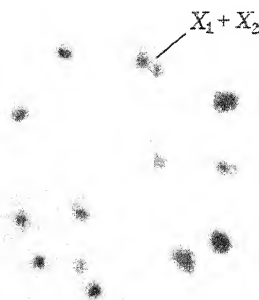


Fig. 16.

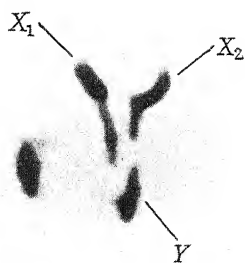


Fig. 17.

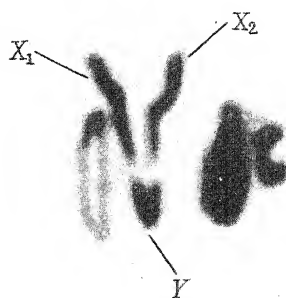


Fig. 18.





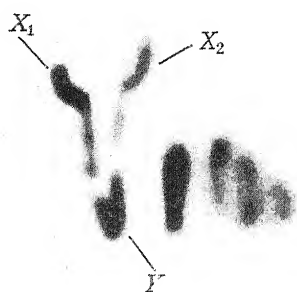


Fig. 19.

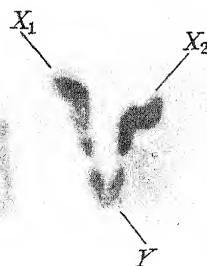


Fig. 20.

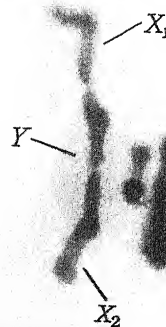


Fig. 21.

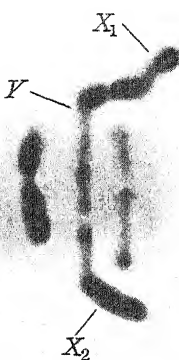


Fig. 22.



Fig. 23.

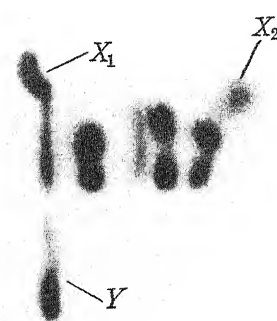


Fig. 24.



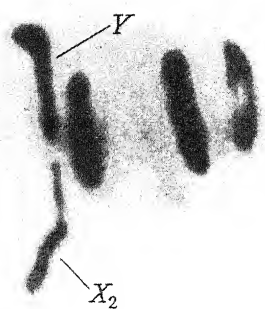


Fig. 25.

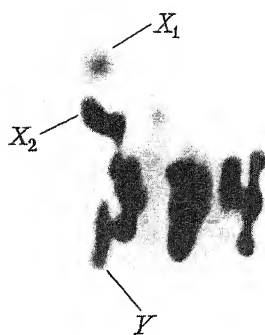


Fig. 26.]

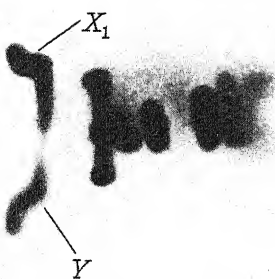


Fig. 27.

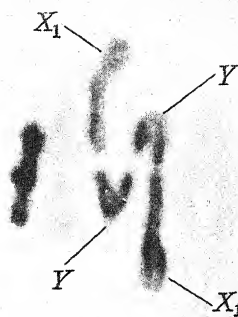


Fig. 28.

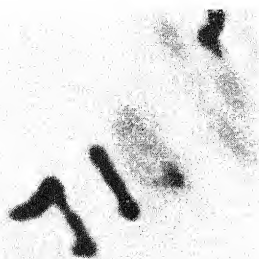


Fig. 29.

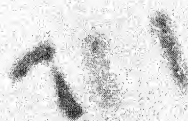


Fig. 30.





Fig. 31.



Fig. 32.



Fig. 33.



Fig. 34.



Fig. 35.



Fig. 36.

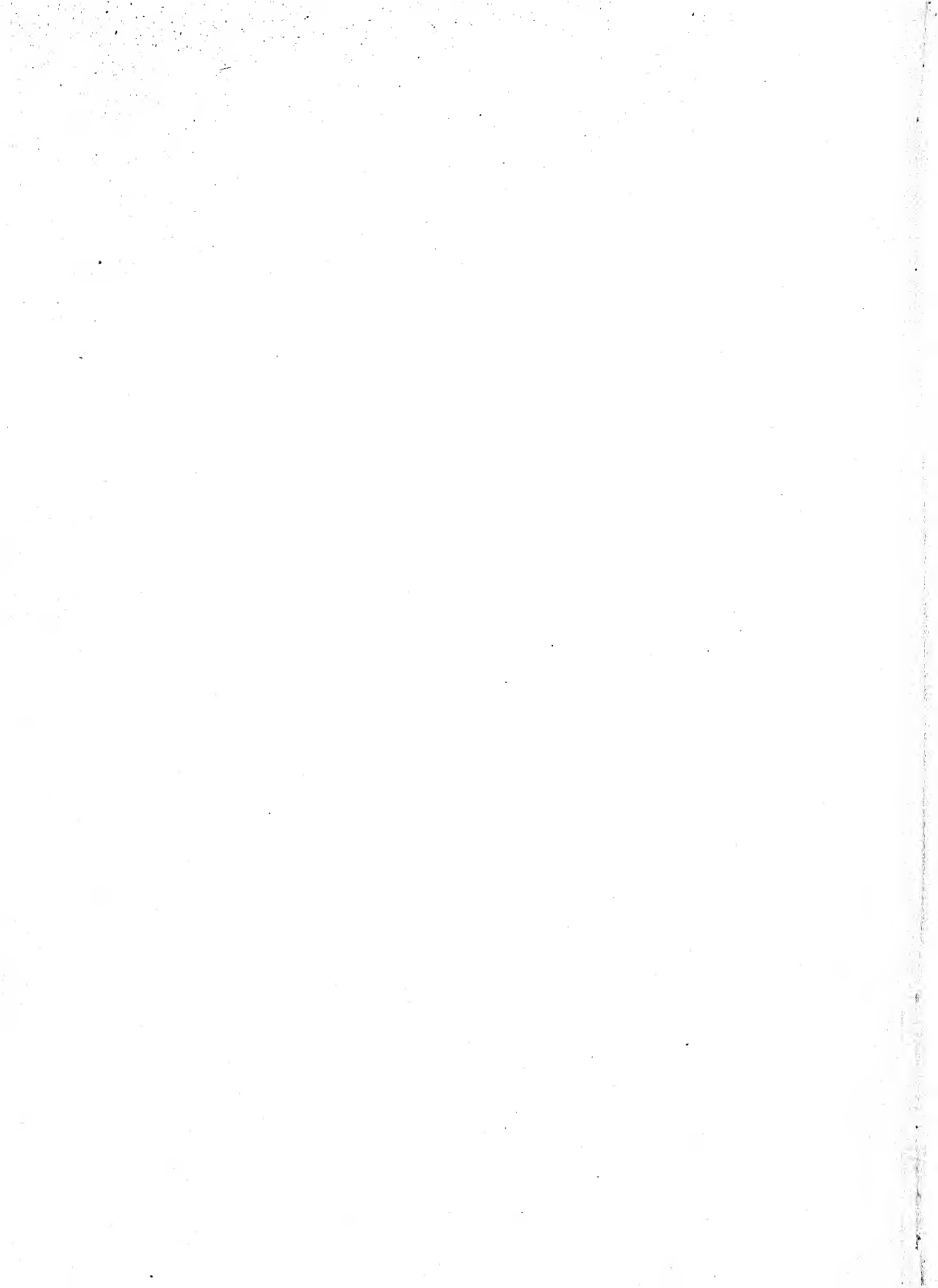


Fig. 5. *Sphodromantis viridis*. Spermatogonial metaphase. Twenty-three mediocentric chromosomes.  $X_1$  and  $X_2$  recognizable, but not distinguishable from one another with certainty.  $Y$  indistinguishable from the autosomes.

Fig. 6. *Paratenodera sinensis*. Spermatogonial metaphase. Twenty-seven mediocentric chromosomes.  $X_1$  and  $X_2$  recognizable, but not distinguishable from one another.  $Y$  indistinguishable from the autosomes.

## PLATE 9

Fig. 7. *Sphodromantis viridis*. Spermatogonial metaphase in side view, showing the heteropycnosis of one chromosome arm (either  $X_1L$  or  $X_2R$ )—labelled "h".

Fig. 8. *Miomantis* sp. First metaphase in side view, showing three autosomal bivalents and the  $X$ -chromosome.

Fig. 9. *Miomantis* sp. First metaphase in polar view, showing seven autosomal bivalents and the  $X$ -chromosome.

Fig. 10. *Ameles abjecta*. First metaphase in polar view, showing fourteen bivalents ( $X$ -chromosome out of focus).

Fig. 11. *Iris oratoria*. First metaphase in polar view, showing twelve autosomal bivalents and the  $X$ .

Fig. 12. *Iris oratoria*. First metaphase in side view, showing three autosomal bivalents and the  $X$ .

## PLATE 10

Fig. 13. *Acontiothespis* sp. First metaphase in polar view, showing seven autosomal bivalents and the  $X$ .

Fig. 14. *Sphodromantis viridis*. First metaphase in polar view, showing ten autosomal bivalents and the  $Y$ -chromosome (seen in optical section).

Fig. 15. *Sphodromantis* sp. First metaphase in polar view, showing twelve autosomal bivalents and the  $Y$ -chromosome (seen in optical section).

Fig. 16. *Paratenodera sinensis*. First metaphase in polar view, showing twelve autosomal bivalents and  $X_1R$  and  $X_2L$  (seen in optical section).

Fig. 17. *Paratenodera sinensis*. The sex trivalent in side view, with an autosomal bivalent on the left.

Fig. 18. *Sphodromantis viridis*. The sex trivalent in side view, with three autosoma bivalents.

## PLATE 11

Fig. 19. *Sphodromantis* sp. The sex trivalent in side view, with four autosomal bivalents on the right.

Fig. 20. *Tenodera aridifolia*. The sex trivalent with an unequal bivalent on the left.

Fig. 21. *Sphodromantis viridis*. Mal-orientation of the sex trivalent. Several autosomal bivalents on the right.

Fig. 22. *Sphodromantis* sp. Mal-orientation of the sex trivalent.

Fig. 23. *Sphodromantis viridis*. Failure of pairing— $\widehat{X_1Y} + X_2$  type (i.e.  $X_2$  as a univalent).

Fig. 24. *Sphodromantis* sp. Failure of pairing— $\widehat{X_1Y} + X_2$  type.

## PLATE 12

Fig. 25. *Sphodromantis* sp. Failure of pairing— $\widehat{X_2Y} + X_1$  type.  $X_1$  not visible (out of focus).

Fig. 26. *Paratenodera sinensis*. Failure of pairing— $\widehat{X_2Y} + X_1$  type.

Fig. 27. *Paratenodera sinensis*. Failure of pairing— $\widehat{X_1Y} + X_2$  type ( $X_2$  out of focus).

Fig. 28. *Tenodera aridifolia*.  $X_1YYX_1$  quadrivalent in a tetraploid spermatocyte.

Fig. 29. *Paratenodera sinensis*. Unequal bivalent at first metaphase.

Fig. 30. *Tenodera aridifolia*. Unequal bivalent at first metaphase.



## PLATE 13

- Fig. 31. *Paratenodera sinensis*. The sex trivalent at the beginning of prometaphase. One of the lateral projections from the ring is out of focus.
- Fig. 32. *Sphodromantis* sp. Prometaphase. Seven autosomal bivalents and part of the sex trivalent in focus.
- Fig. 33. *Acontiothespis* sp. First metaphase in side view, showing the structure of several bivalents. Same cell as that drawn in Text-fig. 2.
- Fig. 34. *Sphodromantis* sp. First metaphase (early stage), showing a bivalent in which the tearing apart process has just begun.
- Fig. 35. *Sphodromantis* sp. A bivalent in a slightly later stage than that shown in fig. 34.
- Fig. 36. *Miomantis* sp. First metaphase, showing the structure of a rod bivalent. Same cell as that drawn in Text-fig. 3c.

# THE EVOLUTION OF THE SEX CHROMOSOMES

## II. THE X-CHROMOSOME IN THE TETTIGONIDAE AND ACRIDIDAE AND THE PRINCIPLE OF "EVOLUTIONARY ISOLATION" OF THE X

By M. J. D. WHITE

University College, London<sup>1</sup>

(With Twelve Text-figures)

### 1. INTRODUCTION

APART from a few sporadic exceptions<sup>2</sup> all the Orthoptera Saltatoria have an  $XO : XX$  sex-determining mechanism. In the males of crickets, Tettigonidae and grasshoppers the sex chromosome is a single unpaired element which is heteropycnotic throughout its whole length at certain stages of meiosis (in the Gryllidae and Acrididae it shows a characteristic reversal of heteropycnosis which is not found in the Tettigonidae—see White, 1940*b*). If heteropycnosis indicates a condition of genetical inertness we must imagine these Orthopteran X-chromosomes as having sex-determining genes or regions distributed at intervals along their length, but we must not expect them to carry a large number of "sex-linked" genes determining characters other than sex.

In the Acrididae the X-chromosome is nearly always telocentric (rod-shaped). The only exceptions to this condition (apart from the  $XY : XX$  species of *Hesperotettix* and *Mermiria* and the  $X_1X_2Y : X_1X_1XX_2$  *Paratytlotropidia*) are in the genera *Circotettix* and *Trimerotropis* (Carothers, 1921; Helwig, 1929; King, 1923). In the Tettigonidae, however, the X is telocentric in many species and mediocentric (V-shaped) in others. In the genus *Drosophila* the same condition is met with, some

<sup>1</sup> This work was carried out at the John Innes Horticultural Institution, Merton. I am indebted to the Director, Dr C. D. Darlington, for the facilities of the Institution after the evacuation of University College owing to the war.

<sup>2</sup> Some of these exceptions ( $XY : XX$  and  $X_1X_2Y : X_1X_1XX_2$  species of Acrididae) have been discussed in a former paper (White, 1940*a*). They have clearly arisen fairly recently from  $XO : XX$  species. The only other Saltatoria which are known to be  $XY : XX$  are *Schizodactylus monstrosus* (McClung & Asana, 1933), *Oecanthus longicauda* (Makino, 1932) and the North European and Rumanian races of *Gryllotalpa vulgaris* (de Winiwarter, 1927; Steopoe, 1939). In none of these cases is it possible at present to determine how the  $XY$  condition is related to the  $XO$  one found in other Orthoptera. It seems probable, however, that all these  $XY$  species have been derived from  $XO$  ones, i.e. that their Y's are "neo-Y's".

species (e.g. *melanogaster*, *virilis*) having telocentric *X*'s while others (e.g. *pseudoobscura*, *miranda*) have mediocentric ones. In this case it is known (Crew and Lamy, 1935; Donald, 1936; McKnight, 1939) that the mediocentric *X* of *pseudoobscura* and *miranda* has arisen from a telocentric *X* by the fusion of the latter with a telocentric autosome or

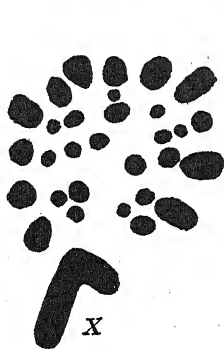


Fig. 1.



Fig. 2.

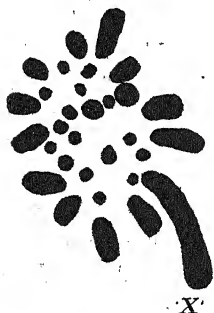


Fig. 3.



Fig. 4.

Figs. 1-4. Spermatogonial metaphases of Phaneropterinae. Fig. 1. *Leptophyes punctatissima* Bosc. Fig. 2. *Microcentrum* sp.<sup>1</sup> Fig. 3. *Insara tolteca* Sauss. Fig. 4. *Insara gracillima* Bruner. Magnification  $\times$  about 3130.

autosomal arm. Thus in the evolution of the *pseudoobscura-miranda* group of species a chromosome arm which was originally present in the diploid condition in both sexes has become haploid in the male.

It is a matter of some interest to determine whether the relationship between the Tettigoniid species with mediocentric *X*'s and those with telocentric ones is the same as that between *D. pseudoobscura* and *D. melanogaster*. Various alternatives are of course possible: (1) the

<sup>1</sup> The number of autosomes in this species was erroneously stated in a former paper to be 16 pairs (White, 1940b); actually it is 15 pairs.

difference between the mediocentric and telocentric  $X$ 's may be merely the result of structural changes within the chromosome which have led to a shift in the position of the centromere, (2) the species with telocentric  $X$ 's may have lost one limb of the  $X$  altogether, (3) the mediocentric  $X$ 's may be the result of "fusion" between an originally telocentric  $X$  and a reduplicated  $X$ -fragment. In one species of *Drosophila* (*D. ananassae*) it is known that a mediocentric  $X$  has arisen by the first method.

In order to determine which, if any, of these hypotheses is true for the Tettigonidae I have collected material of ten species not previously studied by other workers. Together with the forty-six species described in the literature this makes a total of fifty-six species—a fairly representative sample, since it includes members of thirteen subfamilies.

## 2. THE $X$ -CHROMOSOME IN THE TETTIGONIDAE

The method of investigation adopted was to make careful camera-lucida drawings of selected spermatogonial metaphase plates. The lengths of all the chromosomes were measured on the drawing and the length of the  $X$  was then divided by the sum of the lengths of all the autosomes. The values of this fraction  $X/A$  are given in Table 1 and are illustrated graphically in Fig. 10. Great care was taken to secure very clear metaphases lying as nearly horizontal as possible. In this way it was hoped to avoid errors due to the foreshortening of one or more chromosomes. Clearly this is a source of error which it is impossible to eliminate entirely, but there was, in general, good agreement between the results of measurements on different metaphases of the same species. The data given in Table 1 suggest that whatever errors occurred (in the actual drawing or in measuring the drawings) they were too small to affect the general validity of the method employed.

In order to complete the picture I have not confined these measurements to my own drawings, but have worked out the ratio  $X/A$  for all those members of the Tettigonidae whose spermatogonial metaphases have been adequately illustrated by previous authors. Where only one figure had been published for a particular species there was, of course, no means of determining how accurate it was; but in the case of *Amblycorypha* and the three species of *Jamaicana* the results of measuring several figures agree very closely. This suggests that the illustrations of Pearson and Woolsey, at any rate, are highly accurate representations of metaphases with a very small degree of foreshortening. Since we are dealing only with a *ratio* and not with the absolute length of the chromosomes the exact magnification of the figures is not important and the

## The Evolution of the Sex Chromosomes

Table 1. Values of  $X/A$  in the Tettigonidae

		Mean
BRADYPORINAE:		
<i>Callimenus onos</i> (1)	0.087	—
HETRODINAE:		
<i>Eugaster guyoni</i> (2)	0.151	—
PSEUDOPHYLLINAE:		
<i>Jamaicana subguttata</i> (3)	0.115	0.102
	0.102	
	0.090	
<i>J. unicolor</i> (3)	0.107	0.112
	0.117	
	0.096	
<i>J. flava</i> (3)	0.099	0.099
	0.101	
	0.159	
<i>Sathrophyllia</i> sp. (4)		—
MECOPODINAE:		
<i>Mecopoda elongata</i> (4)	0.100	—
TETTIGONINAE:		
<i>Tettigonia viridissima</i> (5)	0.134	0.141
	0.147	
	0.143	
DECTICINAE:		
<i>Decticus albifrons</i> (6)	0.107	—
<i>Platycleis grisea</i> (5)	0.099	0.103
	0.107	
	0.125	
<i>Metrioptera brachyptera</i> (5)	0.122	0.109
	0.096	
	0.122	
<i>Atlanticus pachymerus</i> (5)	0.137	0.129
COPIPHORINAE:		
<i>Neoconocephalus</i> sp. (5)	0.107	0.108
	0.122	
	0.096	
CONOCEPHALINAE:		
<i>Orchelimum</i> sp. (7)	0.164	—
LISTROSCELINAE:		
<i>Hexacentrus mundus</i> (4)	0.107	—
PHANEROPTERINAE:		
<i>Amblycorypha rotundifolia</i> (8)	0.111	0.112
	0.113	
	0.117	
<i>Holochlora</i> sp. (4)	0.197	—
<i>Microcentrum</i> sp. (5)	0.193	0.195
	0.175	
	0.175	
<i>Elimaea securigera</i> (4)	0.191	—
<i>Leptophyes punctatissima</i> (5)	0.186	0.184
	0.173	
	0.150	
<i>Insara gracillima</i> (5)	0.171	0.162
<i>Insara tolteca</i> (5)		—

## References:

- |                          |                            |
|--------------------------|----------------------------|
| (1) Li (1931).           | (5) White (present paper). |
| (2) Favrelle (1936).     | (6) de Winiwarter (1931).  |
| (3) Woolsey (1915).      | (7) King (1924).           |
| (4) Asana et al. (1938). | (8) Pearson (1929).        |

fact that many workers have not thought it worth while to state the magnification of their illustrations is of no consequence. For the same reason it is legitimate to use metaphases of early or late spermatogonial divisions, although the chromosomes are always much shorter in the later divisions.

The species actually studied in the course of this investigation are the following (in the case of the species in brackets no spermatogonial metaphases were found in the material):

Subfamily PHANEROPTERINAE:

*Leptophyes punctatissima* Bosc., England.

*Microcentrum* sp. (probably *rhombifolium* Sauss.), Mexico.

*Insara tolteca* Sauss., Mexico.

*I. gracillima* Bruner, Mexico.

Subfamily DECTICINAE:

*Metrioptera brachyptera* L., England.

*Platycleis grisea* Fabr., England.

(*Pholidoptera griseoptera* de Geer), England.

*Atlanticus pachymerus* Burm., U.S.A.

Subfamily TETTIGONINAE:

*Tettigonia viridissima* L., England.

Subfamily COPIPHORINAE:

*Neoconocephalus* sp. (probably *affinis* or *triops*), Mexico.

Subfamily CONOCEPHALINAE:

(*Conocephalus dorsalis* Thunb.), England.

Subfamily MECONEMINAE:

(*Meconema thalassina* Fabr.), England.

It will be seen that the values of  $X/A$  range from 0.087 (in *Callimenus onos*) to 0.195 (in *Microcentrum*). If we regard the length of the autosomes as being constant, then the  $X$  of *Microcentrum* is more than twice the length of that of *Callimenus*. Even more striking is the difference between *Amblycorypha* ( $X/A=0.112$ ) and *Microcentrum*, since these two genera belong to the same subfamily. It is clear that the variation in length of the  $X$ -chromosome in the Tettigonidae is of the same order of magnitude as that previously observed in some of the subfamilies of the Mantoidea (White, 1941). It remains to be seen whether the observed values of  $X/A$  can be accounted for by any kind of structural rearrangements of the chromosome set which may be inferred to have taken place

in the course of evolution. In order to do this it is clearly advisable to compare species and genera which are closely related from a taxonomic point of view.

### 3. THE SUBFAMILIES OF THE TETTIGONIDAE

(1) BRADYPORINAE. According to Ju Ch'i Li (1931) *Callimemus onos* Pall. has 29 chromosomes, the *X* being telocentric. One pair of autosomes is mediocentric.

(2) HETRODINAE. The spermatogonial chromosomes of *Eugaster guyoni* have been figured by Favrelle (1936). There are 29 of them, all telocentric, the *X* being, as usual, the longest.

(3) EPHIPPIGERINAE. According to Matthey (1939) the somatic set of the male *Ephippigera vitium* consists of 29 chromosomes. The *X* and one pair of autosomes are mediocentric.

(4) PSEUDOPHYLLINAE. Three species of *Jamaicana* were studied by Woolsey (1915). They all have telocentric *X*-chromosomes. The values of  $X/A$  for these three species were determined as 0.099, 0.102, 0.112, but it is doubtful whether the differences between them are significant. *Sathrophyllia* sp. which was studied by Asana *et al.* (1938) was found to have a value of 0.159, which clearly differs significantly from that found in the species of *Jamaicana*. Like *Jamaicana*, *Sathrophyllia* has a telocentric *X*-chromosome.

(5) MECOPODINAE. Only one species of this subfamily has been studied, viz. *Mecopoda elongata*, whose spermatogonial chromosomes are figured by Asana, Makino and Niiyama. It has a mediocentric *X* and  $X/A = 0.100$ .

(6) TETTIGONINAE. *Tettigonia viridissima* with a mediocentric *X* and one pair of mediocentric autosomes (Fig. 8) was found to have  $X/A = 0.141$ .

(7) DECTICINAE. This subfamily is interesting because eight genera have been studied by various authors. In general the members of the Decticinae are characterized by a remarkably uniform chromosome set, consisting of 31 telocentric chromosomes of which the *X* is the longest. *Atlanticus pachymerus*,<sup>1</sup> however, is a notable exception, since it has only 25 chromosomes, of which five (including the *X*) are mediocentric (Fig. 7). The total number of chromosome arms is thus 30. All the chromosomes are very large. The value of  $X/A$  was found to be 0.129 for *Atlanticus*—a figure which is somewhat higher than those found for

<sup>1</sup> The genus *Atlanticus* is probably one of considerable antiquity, since it occurs both in North America and in Asia.

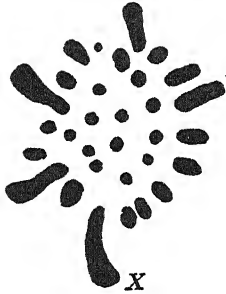


Fig. 5.

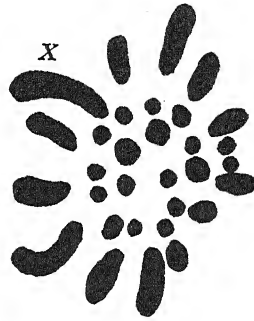


Fig. 6.

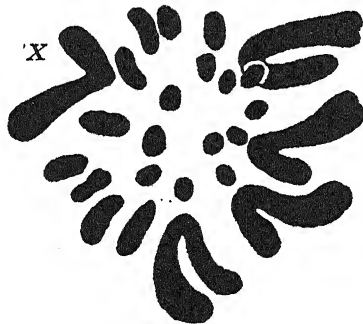


Fig. 7.

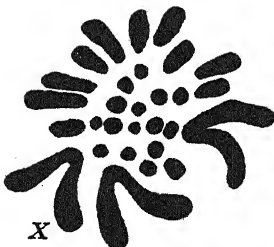


Fig. 8.



Fig. 9.

Figs. 5-9. Spermatogonial metaphases of Decticinae, Tettigoninae and Copiphorinae.  
 Fig. 5. *Platycleis grisea* L. Fig. 6. *Metrioptera brachyptera* Fabr. Fig. 7. *Atlanticus pachymerus* Burm. Fig. 8. *Tettigonia viridissima* L. Fig. 9. *Neoconocephalus* sp.  
 Magnification  $\times$  about 3130.



*Decticus*, *Platypleis* and *Metrioptera* but not high enough to suggest that an autosome had become included in the X of *Atlanticus*. *Pholidoptera griseoptera* was found to have one pair of chromosomes more than the usual number of 31, but since no spermatogonial metaphases were found in the two individuals studied it was not possible to work out a value for  $X/A$ . The X-chromosome is telocentric, as in all the Decticinae except *Atlanticus*.

(8) SAGINAE. The parthenogenetic species *Saga serrata* was found by Matthey (1939) to have a somatic number of 68, of which 12 chromosomes were mediocentric. Since this number is far higher than that of any other Tettigonid studied up till now, Matthey makes the suggestion that the species is a tetraploid. The hypothesis is a very reasonable one, although not definitely proven.

(9) COPIPHORINAE. This and the next subfamily are very closely allied. So far as is known at present all the members of both subfamilies have mediocentric X-chromosomes. An undetermined species of *Neoconocephalus* from Mexico has 23 chromosomes, 9 of them mediocentric (Fig. 9). It was found to have  $X/A = 0.108$ .

(10) CONOCEPHALINAE. Several species of *Conocephalus* have been studied by previous workers. *C. dorsalis* Thunb. was found to have 35 chromosomes, the only mediocentric one being the X. Since no spermatogonial divisions were found in the single animal studied it was not possible to work out a value for  $X/A$ . *Orchelimum* sp. has been studied by King (1924); it was found to have  $X/A = 0.164$ .

(11) LISTROSCELINAE. Two species of *Hexacentrus* were studied by Asana *et al.* (1938) but only in *H. mundus* did they figure the spermatogonial chromosomes. It proves to have  $X/A = 0.107$ .

(12) PHANEROPTERINAE. This is a large subfamily including a great many subtropical and tropical forms. Eight species have been included in Fig. 10, the chromosome sets of four of these having been studied in the present investigation. The values of  $X/A$  range from 0.113 (in *Amblycorypha* sp.) to 0.195 (in *Microcentrum* sp.). An interesting point is the fact that the values of  $X/A$  for the two species of *Insara* (0.162 and 0.171) do not differ significantly from one another.

Of the eight species three have mediocentric X-chromosomes, five have telocentric ones. The three with mediocentric X's have an average  $X/A$  of 0.157, the five with telocentric X's have an average value of 0.158. The difference is clearly not significant and it consequently appears probable that the difference between mediocentric and telocentric X's in this subfamily depends solely on structural rearrangements inside

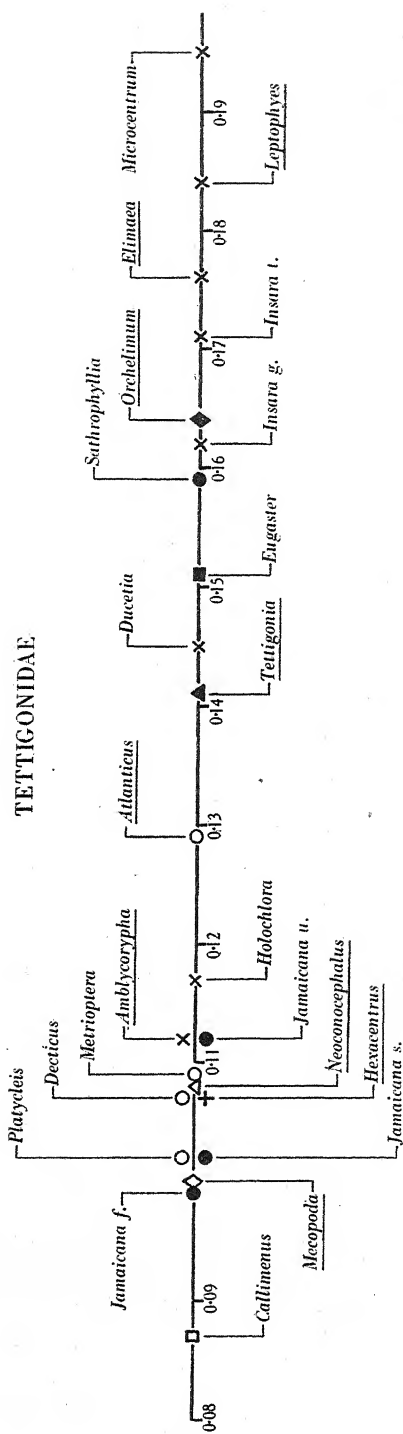


Fig. 10. Values of X/A in twenty-three species of Tettigonidae. The species with medio-centric X's are underlined. Key:  $\square$  = Bradyporinae,  $\blacksquare$  = Heterodinae,  $\times$  = Phaneropterinae,  $\diamond$  = Mecopodinae,  $\bullet$  = Pseudophyllinae,  $\triangle$  = Conocephalinae,  $\Delta$  = Copiphorinae,  $+$  = Listrocelinae,  $\circ$  = Decticinae,  $\blacktriangle$  = Tettigoninae.

the  $X$ , and not on any transference of autosomal chromosome regions to the  $X$  or vice versa.

(13) MECONEMINAE. *Meconema thalassina* has 13 pairs of autosomes, one of which is very large and mediocentric. The  $X$  is one of the medium-lengthed chromosomes and is telocentric. Unfortunately the preparations did not contain any spermatogonial metaphases, so that it was not possible to work out the ratio  $X/A$ .

#### 4. THE $X$ -CHROMOSOME IN THE ACRIDIDAE

The ratio  $X/A$  was also worked out for a number of species of short-horned grasshoppers. The values obtained are given in Table 2 and have

Table 2. Values of  $X/A$  in the Acrididae

TRUXALINAE	
<i>Stenobothrus (Chorthippus) parallelus</i> (1)	0.040
CATANTOPINAE	
<i>Hesperotettix speciosus</i> (2):	
Counting only the "original $X$ " as the $X$ and including the other limb in the autosomes	0.051
Counting both limbs of the $X$ as a single chromosome, but including the $Y$ in the autosomes	0.124
<i>Schistocerca gregaria</i> (3)	0.084
<i>Melanoplus femur-rubrum</i> (3)	0.089
<i>Teratodes monticollis</i> (4)	0.058
OEDIPODINAE	
<i>Chorthippa viridifasciata</i> (3)	0.067
BATRACHOTETRIGINAE	
<i>Haplotropis brunneriana</i> (5)	0.083
PAMPHAGINAE	
<i>Pamphagus marmoratus</i> (6)	0.076
PYRGOMORPHINAE	
<i>Poecilocera picta</i> (7)	0.078
<i>Chrotogonus</i> sp. (8)	0.086
<i>Pyrgomorpha bispinosa</i> (8)	0.099
<i>Aularches miliaris</i> (8)	0.102

(1) Darlington & Dark (1932).

(2) McClung (1917).

(3) White (1940b).

(4) Asana *et al.* (1939).

(5) Li (1931).

(6) Granata (1910).

(7) Asana & Makino (1934).

(8) Rao (1937).

been plotted graphically in Fig. 11. In the case of the Acrididae only published illustrations were used, no species being specially studied for the purpose of this investigation.

The data for the Acrididae are less interesting than those for the Tettigonidae since all the species whose chromosomes were measured have telocentric  $X$ 's. It is however clear on general grounds that the

few species of *Trimerotropis* and *Circotettix* which have mediocentric  $X$ 's have acquired them as a result of structural change within the  $X$  rather than by structural changes involving autosomes at the same time. The species of *Hesperotettix*, *Mermiria* and *Paratytlotropidia* which are  $XY$  or  $X_1X_2Y$  in the male are, however, in a different category. They have mediocentric  $X$ 's as a result of structural changes which simultaneously involved the  $X$  and an autosome.

It will be seen by comparing Figs. 10 and 11 that the values of  $X/A$  are on the whole lower in the Acrididae than in the Tettigonidae, although there is a certain amount of overlapping. The values for the Acrididae are also less variable. This is interesting, since it is well known that chromosome numbers also vary much less in the Acrididae. The three subfamilies Pamphaginae, Pyrgomorphinae and Batrachotetrigininae (which form the "19-chromosome" section of the family) tend to have higher values of  $X/A$  than do the subfamilies Truxalinae, Oedipodinae and Catantopinae (the "23-chromosome" section), although here again there is a certain amount of overlapping.

For the sake of comparison with the data on Tettigonidae and Acrididae the ratio  $X/A$  has been worked out for several of the  $XO$  Mantids described in the previous paper of this series. These values are shown in Fig. 12. The only interesting point about them is the great difference between the very closely allied genera *Empusa* and *Gongylus*. A glance at Pl. 8, figs. 3, 4 of the former paper suggests that the difference is due to the  $X$  of *Gongylus* being very much longer than that of *Empusa*. The  $X$  in *Gongylus* is in fact enormous, not only relative to the length of the autosomes but also in comparison with all the other cellular dimensions (length of the spindles, diameter of the resting nuclei, etc.). If we assume that the autosomal length is the same in *Empusa* and *Gongylus*, then the  $X$  of the latter is longer than that of the former by 70%. As in the case of the Phaneropterinae it seems impossible to avoid the conclusion that in the Empusinae the absolute length of the  $X$  has changed in the course of evolution, as a result of deficiencies, duplications or both.

## 5. DISCUSSION

All the available evidence seems to point to the conclusion that in the Acrididae and Tettigonidae the only interchanges of material between the  $X$ -chromosome and the autosomes which have become permanently established in wild species have been those which converted

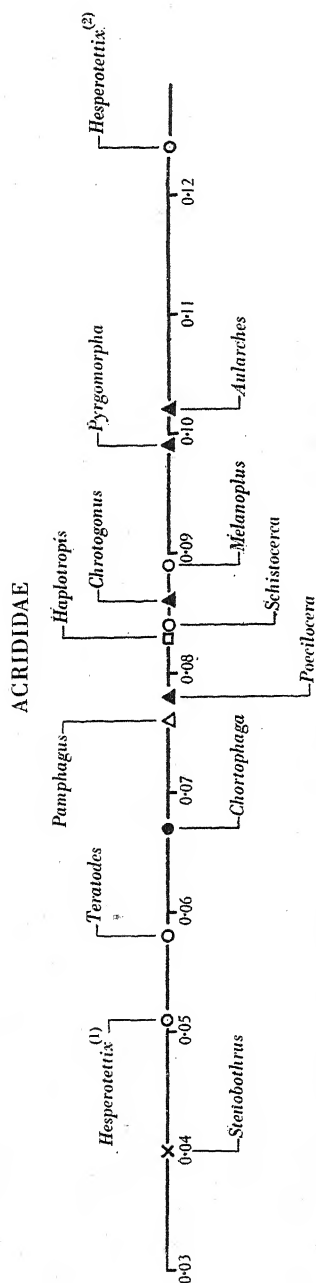


Fig. 11. Values of X/A in thirteen species of Acrididae. Key: O = Truxalinae, ● = Oedipodinae, x = Catantopinae, △ = Pamphaginae, ▲ = Pyrgomorphae, □ = Batrachotetrigenae.

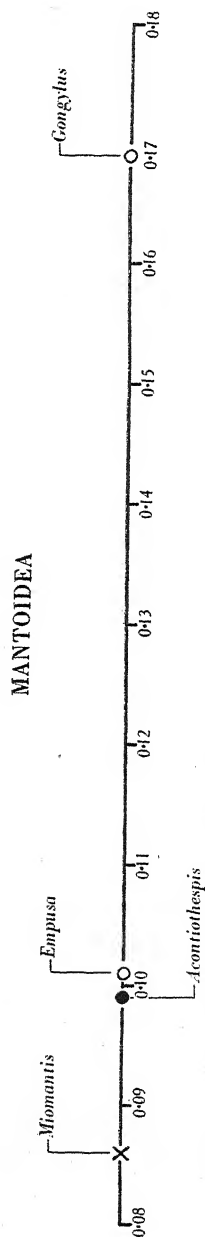


Fig. 12. Values of X/A in four species of Mantoidea. Key: x = Mantinae (*sensu lat.*), ● = Acontiothespinae, O = Empusinae.

the XO system into an XY one (in *Hesperotettix* and *Mermiria*) or into an  $X_1X_2Y$  one (in *Paratytopidia*). Apart from rare exceptions of this nature (involving what Muller (1940) calls *whole-arm transfers*) it seems likely that the X and the autosomes have undergone an independent evolution in the Orthoptera Saltatoria—or at least one which is independent in the sense that no structural changes which involved an exchange of material between the X and an autosome (or a “gift” of material from one to the other) have become established. It is, of course, probable that such rearrangements have taken place in the course of time with a frequency which is of the same order of magnitude as is the case for other translocations; but one can readily understand that they would upset the genic balance of the organism too seriously to permit survival for long. The principle of *structural isolation of the X* (as I should like to call it) seems to be one which—if it is confirmed by future work—will be of the utmost importance in understanding the evolution of the sex-chromosome mechanism.

Apart from a priori reasoning, the evidence for the theory of structural isolation of the X-chromosome (at any rate in the Orthopteroid groups) is threefold:

(1) In the first place we may take it as an established fact that heteropycnosis depends on a particular type of protein framework in the chromosome and that this kind of framework is in some way responsible for (or at any rate associated with) genetical “inertness”. We must consequently expect that a non-heteropycnotic region translocated into or onto a heteropycnotic chromosome would remain non-heteropycnotic and vice versa. Since the X-chromosomes of the Tettigonidae and Acrididae are heteropycnotic throughout their whole length we must assume that such translocations have not occurred. Not only are there no euchromatic segments in the X which might represent autosomal regions included in it, but the converse is also true—the heteropycnotic autosomal regions which are found in many species of Acrididae cannot be derived from the X since they do not show the property of “reversal of heteropycnosis” (White, 1940b).

Two criticisms of this line of argument should be considered. If the translocated region were very small it might perhaps be expected to take on the character (heteropycnotic or otherwise) of the neighbouring material after translocation. Alternatively it might be supposed that autosomal regions translocated to the X might gradually undergo a secular change from euchromatic to heteropycnotic. However, since the autosomal arms which have been included in the sex chromosomes of

*Hesperotettix*, *Mermiria*, *Paratylotropidia* and the Mantinae are still euchromatic we must conclude that such a change, if it occurs, must be extremely slow.

(2) In the second place none of the Tettigonidae and only the three genera of Acrididae already mentioned possess a "neo-Y" chromosome. When a whole autosomal arm is translocated to the *X* of an *XO* species we must expect such a chromosome to arise from the unaltered homologous autosome.

(3) In the third place where a number of related species can be divided into two groups, one with telocentric *X*'s and the other with mediocentric ones, the values of *X/A* do not suggest that a transference of material from the autosomes to the *X* has taken place in the species with mediocentric *X*'s.

There are two possible ways whereby the centromere can spontaneously change its position in the chromosome. Two breaks, one on either side of the centromere, may be followed by reunion of the broken ends the other way round (thus producing a *pericentric inversion*). Alternatively a piece of the chromosome containing the centromere may be inserted into a third break occurring somewhere else along the length of the chromosome (giving rise to a *shift*). It will be noted that both types of rearrangement require that a break should occur on either side of the centromere. In other words, if the centromere were strictly terminal it could neither be shifted nor inverted. It is probable, however, that the chromosomes which we ordinarily regard as telocentric have their centromeres very nearly but not quite terminal.

If we assume that chromosomes are equally likely to break in any region (an assumption which is probably only approximately correct) it is clear that the likelihood of the centromere changing its position as a result of a shift or an inversion is greatest when it is median and falls off in direct proportion to its distance from the middle (the distance being measured by the number of possible breakage points). Thus the telocentric (really subterminal) position should be the most stable one. This argument is, of course, a purely theoretical one and does not take into consideration the fact that some types of rearrangement will have a better chance of survival than others. But it does help one to understand why in many groups only telocentric chromosomes are found. Where a large number of chromosomes have suddenly become mediocentric as a result of internal rearrangements (pericentric inversions or shifts)—as has apparently happened in *Circotettix verruculatus* (Helwig, 1929) and *Trimerotropis* spp. (King, 1923), then it is likely that some

special cause must have been at work, favouring either the production or the survival of such rearrangements.

When once the centromere has changed its position in the chromosome the new arrangement will give rise to duplications and deficiencies by crossing-over with the original chromosome. It is possible that such duplications and deficiencies may be the explanation of the variation in length of the *X* which we have noted in groups such as the Phaneropterinae and the Empusinae. There is, however, no reason to suppose that this is the only way in which deficiencies and duplications have occurred—they may equally well have arisen in chromosomes where no previous change in the position of the centromere has taken place.

In Table 3 the genera of the Tettigonidae have been divided into four groups:

- (1) those with a telocentric *X* and all autosomes telocentric;
- (2) those with a telocentric *X* and some autosomes mediocentric;
- (3) those with a mediocentric *X* and all autosomes telocentric;
- (4) those with a mediocentric *X* and some autosomes mediocentric.

Table 3. *X*-chromosome

Telocentric		Mediocentric
AUTOSOMES		
<i>All telocentric</i>		
<i>Eugaster</i>		<i>Amblycorypha</i>
<i>Saltrophyllia</i>		<i>Leptophyes</i>
<i>Decticus</i>		<i>Hexacentrus</i>
<i>Metrioptera</i>		( <i>Conocephalus</i> )
<i>Gampsocleis</i>		
<i>Pholidoptera</i>		
<i>Anabrus</i>		
<i>Ducetia</i>		
<i>Holochlora</i>		
<i>Microcentrum</i>		
<i>Insara</i>		
<i>Phaneroptera</i>		
( <i>Jamaicana</i> )		
12½		3½
<i>Some mediocentric</i>		
<i>Callimenus</i>		<i>Ephippigera</i>
<i>Meconema</i>		<i>Mecopoda</i>
( <i>Jamaicana</i> )		<i>Tettigonia</i>
		<i>Atlanticus</i>
		<i>Elismaea</i>
		<i>Isotima</i>
		<i>Neoconocephalus</i>
		<i>Homoeocoryphus</i>
		<i>Orchelimum</i>
		( <i>Conocephalus</i> )
2½		9½



Most of the data from which this table was constructed were derived from the paper of Asana *et al.* (1938). A few genera in which several species have been investigated fell into more than one group; these have been placed in brackets. It will be seen that twenty-one genera fall into groups 1 and 4, only five into categories 2 and 3 (two genera fall into more than one group). This would seem to indicate that there is a tendency for the larger autosomes to be the same shape as the  $X$ .<sup>1</sup> It is possible that there is some mechanical advantage in having several chromosomes mediocentric if one is so. However, the mediocentric autosomes of the Tettigonidae have probably arisen in a different way from the mediocentric  $X$ 's—namely, by "fusion" of originally distinct telocentric autosomes. This hypothesis is very strongly supported by a study of chromosome numbers in the group: one finds that wherever one or more mediocentric autosomes is present the chromosome number is reduced (compare *Tettigonia* and *Atlanticus* with the "normal" Decticinae and *Neoconocephalus* with *Conocephalus*).

## 6. CONCLUSIONS

1. Structural changes simultaneously involving the  $X$ -chromosome and an autosome have either not occurred at all in the Tettigonidae or at any rate have occurred far more rarely than those involving the autosomes alone. The  $X$  is consequently in a state of "evolutionary isolation". The differences between telocentric and mediocentric  $X$ 's when these occur in closely related genera are due solely to internal rearrangements in the  $X$  and not to the mediocentric ones having acquired an extra arm from the autosomes (as has happened in *Drosophila*).

2. These conclusions apply also to the Acrididae and the Mantoidea with the exception of the few genera where  $XY$  or  $X_1X_2Y$  species have arisen from  $XO$  ones as a result of "whole arm transfers" to the  $X$ .

3. In those subfamilies of Tettigonidae and Mantoidea where some of the species have  $X$ 's of very different absolute length we must assume that duplications and deficiencies have arisen in the  $X$  in the course of its evolution. Such duplications and deficiencies may possibly have been beneficial to the species if they readjusted the genic balance, upon which sex determination depends, to a slightly more advantageous equilibrium position.

<sup>1</sup> The smaller autosomes are never mediocentric in the Tettigonidae.

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## AN ALLELIC SERIES IN *COLEUS*<sup>1</sup>

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(With Plate 14)

VARIATION in the leaf coloration of *Coleus* is one of the most obvious characteristics of this garden plant to attract the attention of geneticists. The usefulness of *Coleus* as genetic material is enhanced by the production of large numbers of seed and the fact that it can be propagated vegetatively.

During the past three years, investigations concerning genetically determined variation in leaf coloration in several clones of *Coleus* have been in progress in the Genetics Laboratory, Ohio State University. One of these clones, known as the PURPLE clone, arose from recombination in hybrid seed, and has been propagated vegetatively at this institution for about five years. A second, GOLDEN BEDDER, a well-known commercial clone, was purchased from a commercial greenhouse, and has a history of about ten years of vegetative propagation at this university.

The purpose of this paper is to survey the evidence collected prior to and since 1938 and the genetically conclusive evidence collected since 1938 regarding the relationships of several factors in these two clones of *Coleus*.

Presumptive evidence (Boye & Rife, 1938) indicated that the PURPLE clone is heterozygous for two independent pairs of factors. The dominant factor **P** of the first pair was assumed to be associated with the appearance of leaves which are *purple* in colour (Pl. 14 A) when viewed from either the upper or lower surface; its allele **p** was presumably associated with the appearance of leaves which show a reddish brown area on the upper surface with a narrow green border and a green lower surface. The latter condition was designated as *pattern* leaves (Pl. 14 B, C). The dominant factor **I** of the second pair of factors was assumed to be associated with the appearance of leaves which in the green areas are of a blue-green colour (Pl. 14 B, E); its allele **i** was presumed to be associated with the appearance of leaves which in the green areas are of a yellow-green colour (Pl. 14 C, D). These two conditions are now designated as *intense*

<sup>1</sup> "Genetic studies of *Coleus*, III."

and *dilute* respectively. The dominant factor of the first pair was reported to be epistatic to the second pair of factors.

The second clone, known as GOLDEN BEDDER, was reported (Boye & Rife, 1938) to be presumably heterozygous for one pair of factors. The dominant allele  $p^G$  was assumed to be associated with the appearance of leaves which are *green* in colour throughout (Pl. 14 D); its recessive  $p$  was presumably associated with the appearance of leaves which have an almost scarlet area on the upper surface surrounded by a narrow green border and a green lower surface (Pl. 14 C). This condition was designated as *pattern*.

A thorough analysis of the data obtained by selfing the PURPLE clone is presented in Table 1, in which a  $\chi^2$  value and its probability are given for each seed sample. In order to insure the applicability of the  $\chi^2$  analysis, a given seed sample was obtained when necessary by combining several smaller seed samples so as to make the smallest expected number approximately ten (Mather, 1938). In addition, a total  $\chi^2$  value and its probability are given for the series of seed samples; deviation and heterogeneity  $\chi^2$  values and their probabilities are given as the last analysis of this set of data. These analyses indicate clearly the a priori validity of the assumptions concerning the genetic basis for the phenotypic variation observed in the selfed progenies of the PURPLE clone. Genetically conclusive evidence concerning the factors presumably involved and their relationship is given in a similar presentation in Table 2. Plants of the PURPLE clone were used as female parents in these crosses.

An intensive analysis of the data obtained by selfing the GOLDEN BEDDER clone is given in Table 3. This shows the assumption concerning the genetic basis for the phenotypic variation in the GOLDEN BEDDER clone to be valid.

The foregoing analyses (Tables 1-3) raise several interesting questions. Does the factor for *pattern* leaves in the PURPLE clone occupy the same locus as the factor for *pattern* leaves in the GOLDEN BEDDER clone? Is the appearance of leaves of a yellow-green colour in their green areas (*dilute*) in the PURPLE and GOLDEN BEDDER clones ascribable to the same factor? What is the relationship of the factor for *purple* leaves in the PURPLE clone and the factor for *green* leaves in the GOLDEN BEDDER clone? Analysis of the data obtained by crossing the PURPLE and GOLDEN BEDDER clones is presented in Table 4. The first seed sample was obtained by using plants of the PURPLE clone as female parents; the second resulted from crosses in which plants of the GOLDEN BEDDER

clone were used as female parents. This analysis shows that the following assumptions concerning the relationships of the factors in the PURPLE clone to the factors in the GOLDEN BEDDER clone are valid: (1) the factor for *purple* leaves in the PURPLE clone is an allele of the factor for *green*

Table 1. *Analysis of data obtained by selfing the PURPLE clone*

Seed sample	Purple (Pl. 14 A)	Pattern, intense (Pl. 14 B)	Pattern, dilute (Pl. 14 C)	$\chi^2$	Degrees of freedom	Probability
1	134	34	15	1.188	2	0.7-0.5
2	109	27	11	0.384	2	0.9-0.8
3	106	26	12	1.074	2	0.7-0.5
4	95	24	11	1.136	2	0.7-0.5
5	213	56	13	1.408	2	0.5-0.3
6	129	32	7	0.674	2	0.8-0.7
7	102	29	13	2.258	2	0.5-0.3
Totals	888	228	82	8.122	14	$p=0.9-0.8$

Deviation  $\chi^2=0.852$ ; D.F.=2;  $p=0.7-0.5$ . Heterogeneity  $\chi^2=7.270$ ; D.F.=12;  $p=0.9-0.8$ .

Table 2. *Analysis of data obtained by backcrossing the PURPLE clone*

Seed sample	Purple (Pl. 14 A)	Pattern, intense (Pl. 14 B)	Pattern, dilute (Pl. 14 C)	$\chi^2$	Degrees of freedom	Probability
1	72	30	31	0.925	2	0.7-0.5
2	78	44	46	0.905	2	0.7-0.5
3	45	17	19	1.098	2	0.7-0.5
Totals	195	91	96	2.928	6	$p=0.9-0.8$

Deviation  $\chi^2=0.299$ ; D.F.=2;  $p=0.9-0.8$ . Heterogeneity  $\chi^2=2.629$ ; D.F.=4;  $p=0.7-0.5$ .

Table 3. *Analysis of data obtained by selfing the GOLDEN BEDDER clone*

Seed sample	Green, dilute (Pl. 14 D)	Pattern, dilute (Pl. 14 C)	$\chi^2$	Degrees of freedom	Probability
1	60	14	1.460	1	0.3-0.2
2	17	8	0.652	1	0.5-0.3
3	44	7	3.456	1	0.1-0.05
4	47	23	2.304	1	0.2-0.1
5	46	10	1.524	1	0.3-0.2
6	30	16	2.348	1	0.2-0.1
7	48	18	0.180	1	0.7-0.5
8	52	20	0.296	1	0.7-0.5
9	64	20	0.064	1	0.9-0.8
10	38	10	0.444	1	0.7-0.5
Totals	446	146	12.728	10	$p=0.3-0.2$

Deviation  $\chi^2=0.036$ ; D.F.=1;  $p=0.9-0.8$ . Heterogeneity  $\chi^2=12.692$ ; D.F.=9;  $p=0.2-0.1$ .

leaves in the GOLDEN BEDDER clone; (2) the factor for yellow-green (*dilute*) coloration of leaves in their green areas occupies the same locus in the PURPLE and GOLDEN BEDDER clones.

As a final test of these assumptions the new *grey* phenotype (Pl. 14 F, G) was both selfed and test-crossed. Data obtained by selfing the new *grey*

phenotype are analysed in Table 5, and an analysis of data obtained by crossing this phenotype (genotype **Pp<sup>G</sup>Ii**) with plants of the genotype **ppii** (Pl. 14 C) is given in Table 6. The latter was the male parent in these crosses. As expected, the selfed segregation of the new *grey*

Table 4. *Analysis of data obtained by crossing the PURPLE and GOLDEN BEDDER clones*

Seed sample	<i>Grey</i> (Pl. 14 F, G)	<i>Purple</i> (Pl. 14 A)	<i>Green</i> (Pl. 14 D, E)	<i>Pattern</i> (Pl. 14 B, C)	$\chi^2$	Degrees of freedom	Probability
1	14	9	11	14	1.499	3	0.7-0.5
2	13	10	11	8	1.238	3	0.8-0.7
Totals	27	19	22	22	2.737	6	$p=0.9-0.8$

Deviation  $\chi^2=1.466$ ; D.F.=3;  $p=0.7-0.5$ . Heterogeneity  $\chi^2=1.271$ ; D.F.=3;  $p=0.8-0.7$ .

Table 5. *Analysis of data obtained by selfing the new grey phenotype*

Seed sample	<i>Purple</i> (Pl. 14 A)	<i>Grey</i> (Pl. 14 F, G)	<i>Green</i> (Pl. 14 D, E)	$\chi^2$	Degrees of freedom	Probability
1	12	26	18	1.572	2	0.5-0.3
2	10	26	13	0.551	2	0.8-0.7
3	9	15	6	0.600	2	0.8-0.7
Totals	31	67	37	2.723	6	$p=0.9-0.8$

Deviation  $\chi^2=0.541$ ; D.F.=2;  $p=0.8-0.7$ . Heterogeneity  $\chi^2=2.182$ ; D.F.=4;  $p=0.8-0.7$ .

Table 6. *Analysis of data obtained by crossing the grey phenotype (genotype Pp<sup>G</sup>Ii) with pattern, dilute (ppii) plants*

Seed sample	<i>Purple</i> (Pl. 14 A)	<i>Green, intense</i> (Pl. 14 E)	<i>Green, dilute</i> (Pl. 14 D)	$\chi^2$	Degrees of freedom	Probability
1	34	13	12	1.135	2	0.7-0.5
2	19	13	12	0.864	2	0.7-0.5
3	18	5	8	1.386	2	0.5
4	11	8	7	0.692	2	0.8-0.7
5	17	5	6	1.357	2	0.7-0.5
Totals	99	44	45	5.434	10	$p=0.9-0.8$

Deviation  $\chi^2=0.542$ ; D.F.=2;  $p=0.8-0.7$ . Heterogeneity  $\chi^2=4.892$ ; D.F.=8;  $p=0.8-0.7$ .

phenotype took the form of a 1 : 2 : 1 phenotypic distribution, and the test-cross, a 2 : 1 : 1 distribution of *purple*, *green* and *intense*, and *green* and *dilute* phenotypes.

#### SUMMARY

Rigorous examination of data obtained by (1) selfing the PURPLE and GOLDEN BEDDER clones, (2) test-crossing the PURPLE clone, (3) crossing the PURPLE and GOLDEN BEDDER clones, and (4) selfing and test-crossing the new *grey* phenotype thus obtained indicate clearly the validity of the following conclusions (cooperatively illustrated by Rife & Boye, 1940) concerning the genetic basis for the phenotypic variation thus far observed in the PURPLE and GOLDEN BEDDER clones of *Coleus*:

(1) Two independent loci have been specified: one is occupied by the alleles **P**, **p<sup>g</sup>**, and **p**; the other is occupied by the factors **I** and **i**.

(2) Plants of the genotype **PpI-** and **Ppii** have leaves which are *purple* in colour (Pl. 14 A), thus indicating the dominance of **P** to **p** and the epistasis of **P** to the independent pair of factors **I** and **i**, factors for blue-green (*intense*) and yellow-green (*dilute*) coloration of leaves in their green areas respectively.

(3) The genotype **p<sup>g</sup>pI-** results in leaves which are *green* and *intense* (Pl. 14 E), thus illustrating the dominance of **p<sup>g</sup>** to **p** and of **I** to **i**.

(4) The leaves of plants of the genotype **p<sup>g</sup>pii** are *green* and *dilute* (Pl. 14 D).

(5) Plants of the genotype **ppI-** have leaves which are designated as *pattern* and *intense* (Pl. 14 B).

(6) Plants of the genotype **ppii** have leaves which are known as *pattern* and *dilute* (Pl. 14 C).

(7) Plants of the genotype **Pp<sup>g</sup>I-** have leaves which are called *grey* and *intense* (Pl. 14 F), thus indicating a phenotypic blending of the alleles **P** and **p<sup>g</sup>**, and the reduction of the phenotypic epistasis of the factor **P** to the factor **I**.

(8) Plants which genotypically are **Pp<sup>g</sup>ii**, are phenotypically *grey* and *dilute* (Pl. 14 G).

The terms *intense* and *dilute* are used advisedly and constitute a correction for an earlier publication (Boye & Rife, 1938) in which the terms *intense chlorophyll* and *dilute chlorophyll* were used. At present, an attempt is being made to interest plant anatomists and physiologists in further determining the nature of the characters associated with the genetic factors thus far specified in *Coleus*, particularly as has been done with respect to flower colour in several species of ornamental plants (Scott-Moncrieff, 1936).

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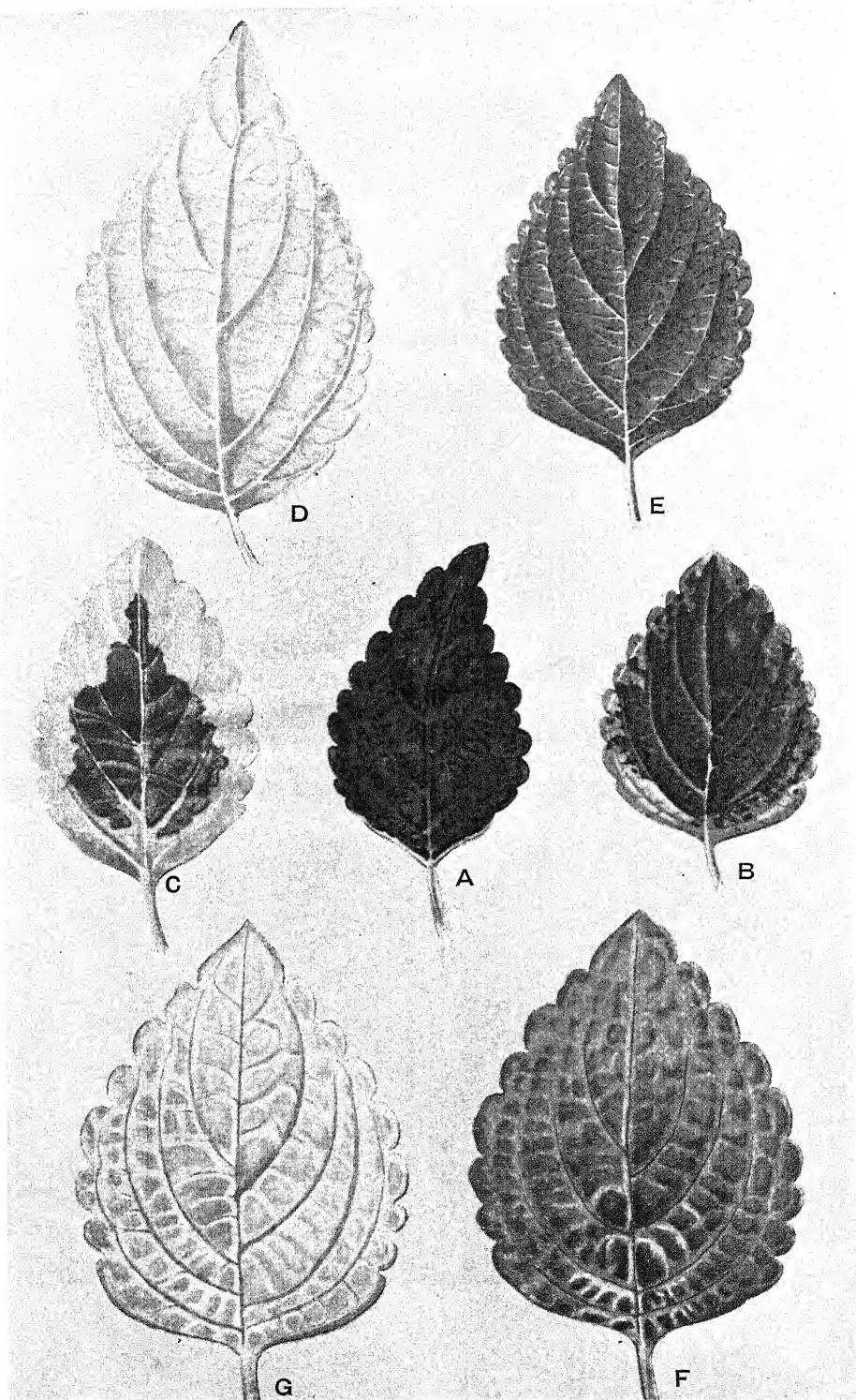
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## EXPLANATION OF PLATE 14

- |   |  |   |  |
|---|--|---|--|
| A | Purple— <b>PpI-</b> or <b>Ppii.</b>    | E | Green intense— <b>p<sup>a</sup>pI-</b> . |
| B | Pattern intense— <b>ppIi.</b>          | F | Grey intense— <b>Pp<sup>a</sup>I-</b> .  |
| C | Pattern dilute— <b>ppii.</b>           | G | Grey dilute— <b>Pp<sup>a</sup>ii.</b>    |
| D | Green dilute— <b>p<sup>a</sup>pii.</b> |   |  |





# GENE RELATIONS AND SYNTHETIC PROCESSES

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THE gene is a unit of segregation, crossing-over and mutation. A unit of segregation may not consist of exactly the same amount of chromosome material as a unit of crossing-over or mutation, but for most purposes it is convenient and satisfactory to consider all three as the same thing. Recently, a fourth aspect of the gene has been recognized: that concerned with development. Since there appears to be no limit to the number or variety of characters which may show the results of Mendelian segregation, it follows that every developmental process must have its controlling genes.

Ideally, therefore, it might be possible to analyse the synthesis of a particular plant or animal product by removing, one by one, the genes which govern the intermediate steps in its manufacture. Such a procedure is at present impossible because, first, we are aware of only a small proportion of the genes controlling any given synthesis (since only those genes which have mutated can be discovered), and secondly, genetical interaction would make a direct analysis impossible; the function of a single gene cannot be isolated from that of others affecting related processes. A third but minor difficulty is that it is not practicable to remove a gene without substituting another allelomorph, which may itself have some influence on the same process as the original gene.

There are therefore insuperable difficulties which prevent us from learning the details of a synthesis by removing, one by one, the genes which control it, and we must resort to indirect methods. The present article is an attempt to use as indicators of the direction of synthetic processes the relationships which one gene bears to another. If two genes are allelomorphous, they may be related in that one is a wild type and one a mutant allelomorph, or that one is dominant and the other recessive (though it is quite realized that these terms have no meaning for some genes). If the two genes are not allelomorphous, one may be epistatic to the other. Thus there are three ways in which two genes may be related: by mutation and dominance if they are allelomorphous, by epistasis if they are not.

In order to test any hypothesis on the physiological significance of

these relationships, it would be desirable to have available some synthetic process which is carried through to varying degrees of completion in different species and different varieties of the same species. Thus suppose a substance *A* is an intermediate product in the synthesis of another substance *B*. Then, one would require some species in which the wild type (as defined later) contained *A*, with mutants containing *B*; and some species where the wild type contained *B* and mutants *A*. It would then be possible to gain an idea of the relationships of the genes associated with *A* and *B* in the different species.

The nearest approach to this ideal is probably offered by the anthocyanin pigments: there is an abundance of wild type and mutant forms, and the pigments in the mutants of some species are the same as those in the wild types of others. Information is, however, lacking on the synthesis in living plants of anthocyanins, though several of them have been synthesized in the laboratory.

## 1. GENERAL CONSIDERATIONS

### (a) *Mutation*

It will be necessary first of all to define the terms *wild type* and *mutant*, since they have considerable importance for the discussion which follows. Gene mutations can be roughly sorted into three groups: (1) Those which produce a striking change from an easily recognized standard type to a new form which occurs rarely, if at all, in wild populations. It is of course this kind of mutation which is usually studied in Mendelian experiments, and the notion of a wild-type allelomorph is obvious. Of the total population of a species, the great majority of individuals contain the wild-type gene, and only a small proportion the mutant. (2) Mutations concerned with sharply discontinuous characters like self-sterility in plants, or colour patterns in *Lebistes* or in polymorphic butterflies. Here no one type is any more standard than another, and it is idle to speak of a wild-type or mutant gene. (3) "Polygenes" (as they are termed by Mather, 1941), i.e. genes whose effect is individually slight, but cumulative on some metric character. Though little work has been done with this kind of variation, it is already clear that different populations of a single species may vary in the allelomorphs of these genes which are carried, even though in appearance individuals in different populations may not be markedly different. The term "wild-type allelomorph" has no meaning for this third type of mutation

either. Hence in the succeeding discussion only the first group will be considered.

Since most of the allelomorphs in wild populations have survived a rigorous selection, it follows that random mutation to other allelomorphs usually results in the production of deleterious genes, giving less viable phenotypes (as often pointed out, for example, by Fisher (1928), Wright (1929), Muller (1932)). Apart from the matter of fitness, however, the question I shall consider is this: Are mutant allelomorphs likely to lead to the formation of chemical substances which are intermediate products in the synthetic processes taking place in the wild type, or to cessation of the development of some organ at an incomplete stage? If so, we shall have an important method for determining the intermediate steps of such processes.

Any given stage in a series of biochemical reactions can only be achieved if a number of conditions are favourable; for example, the right raw materials must be present in the right concentrations; the *pH* of the medium must be suitable; possibly some catalyst must be present. These different conditions are controlled by different genes, which are known often to be highly specific in their effects. Even quite short steps may be expected to be controlled by a number of genes and, if one such gene is lacking, some condition is made unfavourable for the particular reaction, which is therefore stopped. The existence of a unifactorial difference between two forms does not imply that *only* one gene is required to convert one into the other. Thus in maize, the difference between green- and yellow-leaved plants is unifactorial, but it is well known that many genes must be present together if chlorophyll is to be synthesized. Similarly with quite small differences, like those between anthocyanins, it must not be assumed that only one gene is necessary to convert a pelargonidin derivative into a cyanidin derivative, just because the two pigments segregate in a 3 : 1 ratio. The pelargonidin type used already contains all except one of the genes necessary for the formation of delphinidin.

The following hypothetical example will show how the relative stability of two characters is related to their development. A pigment has two forms: a simpler, *A*, and a more complex, *B*, i.e. in the synthesis of *B*, *A* is temporarily formed as an intermediate product. The difference between the two forms is controlled by three genes *X*, *Y*, *Z*, all of which must be present if *B* is to be produced, and none of which are concerned with the formation of *A*. Species containing *B* in the wild can produce *A* mutants if any one of the genes *X*, *Y* or *Z* mutates to its recessive

allelomorph; but species containing *A* in the wild have the constitution **xyz**, and all three must simultaneously mutate if a *B* mutant is to be produced. This latter is so unlikely that, in effect, mutation from the wild type will always be in the direction  $B \rightarrow A$ .

It must, however, be admitted that there are some reactions so balanced that, if one substance is partially or completely inhibited, another substance, derived from the same raw materials as the first, is produced in increased quantity. This happens, for example, with anthocyanins and anthoxanthins (see Lawrence & Scott-Moncrieff, 1935). But in such cases one must remember that the alternative substance was already present, though in reduced amount, under the original conditions, and the main lines of its synthesis were already laid down. No new system had to be created.

Another possible objection to the view that mutations away from the wild type will more usually lead to the formation of simpler substances (i.e. those requiring fewer stages for synthesis) than to more complex substances, is that in *Drosophila* and other well-studied organisms there are quite a number of "gene-suppressors" known, e.g. of vermilion, sable, black and purple (see Schultz & Bridges, 1932). These suppressor genes when superimposed on the corresponding mutants produce a fly in appearance like the wild type. Here, it might be argued, are mutations in a "building-up" direction. This is, however, not a valid objection, because such suppressors have only to replace the function of the single gene which they are "suppressing" (to use the *Drosophila* terminology); they do not themselves control all the stages necessary for the formation of the wild-type eye pigment from the mutant eye pigment.

The above argument is concerned with mutation away from wild-type allelomorphs. This should not be confused with mutation rates to and from particular allelomorphs. Natural mutation rates have been so little investigated that it is impossible to know whether the more frequently occurring mutations are associated with any particular kind of physiological change. Significant differences in mutation rates are known, for instance, at the white locus in *D. melanogaster* (see Timoféeff-Ressovsky, 1937, p. 40), but these are presumably dependent on the physical structure of the gene and not related to the processes which it brings about. There is no evidence against this view, but very little for it.

There are therefore three facts to be considered when assessing the physiological significance of the most frequently occurring direction of mutation of genes having an obvious wild-type allelomorph:

(1) The wild-type allelomorph has been selected for a particular environment, and differs from all other allelomorphs in this respect.

(2) Most synthetic processes are controlled by many genes acting in collaboration, so that, if one fails, a reaction will be stopped. It is unlikely to proceed in a different direction unless an alternative reaction already takes place to a lesser extent under the original conditions.

(3) There are significant differences in the mutation rates of individual genes, but little is known of the laws governing these differences.

Applying these conclusions to the data available, it is at once obvious that wild-type allelomorphs more frequently mutate in a "destructive" than in a "constructive" direction (a fact which was at one time thought to disprove the importance of mutation in evolution). For example, a rapid search through a seed catalogue will reveal that a high proportion of flowering plants whose wild types contain anthocyanin pigments in the flowers have given rise to white varieties; but species which have a white-flowered wild type only rarely give pigmented varieties.

Muller (1932) has shown that the great majority of mutant genes in *D. melanogaster* are (in his terminology) hypomorphs or amorphs. Hypermorphs and neomorphs arise only rarely. This gives some support to the view that genes most frequently mutate from a "more active" to a "less active" state, since several doses of a hypomorphic mutant gene act like a single dose of the wild-type allelomorph. A distinction should, however, be drawn between "activity" as measured by the number of doses of a gene necessary to produce a given effect, and as measured by the amount of substance finally produced.

It is also necessary to distinguish between quantitative and qualitative variations. By quantitative is meant the reduction in amount (or total suppression) of a substance, by qualitative the substitution of a different substance, owing to the interruption of a synthesis and the accumulation of an intermediate product. Data on such qualitative variations form the second part of this paper.

#### (b) Dominance

Dominance has been interpreted on a physiological basis by Haldane (1930), Goldschmidt (see 1938), Muller (1932), Wright (1929) and others. Two postulates are necessary: (1) a recessive gene is less active than its dominant allelomorph, and (2) one dose of a dominant gene is as effective as two. (Alternatively, if the dominant gene is less active than the recessive, one dose of the recessive is below the threshold value necessary to produce any effect, two doses being essential.) This restatement in



physiological terms of the phenomenon of dominance does not necessarily conflict with the view of Fisher (see 1931), that dominance is one of the characters for which the wild form of a species has been selected. The actual mechanism of the process of selection is, however, not clear—whether it is by Fisherian dominance modifiers, whether by a selection of allelomorphs as proposed by Haldane (1930), or whether selection acts, not on heterozygotes, but directly on the homozygous wild type, as Muller (1932) suggests, “to provide a margin of stability and security”. In any case there is a selection of wild-type allelomorphs both for their desirable phenotypic effects and for dominance. In some plants and animals, however, this selection has not been so successful, and in these there is a considerable proportion of non-recessive mutants. It is important to realize that, in these organisms, the dominant mutations do not differ *physiologically* from the recessive ones; the disorganizations commonly produced by mutant genes occur just as much when they are dominant as when they are recessive. For example, in *Primula sinensis* (de Winton & Haldane, 1935), among the “dominant” (or incompletely recessive) mutants are **D** and **Ye**, the former suppressing anthocyanin in the flowers, the latter reducing the amount of chlorophyll in the leaves. Similarly, Howard (1940) has recently described two dominant mutants in *Armadillidium vulgare*, both containing less of some body pigment than the common type. The fowl is another animal having a high proportion of non-recessive mutants, and among them deficiencies of some substance or part of the body are found as commonly as in any more normal organism where the mutants are mostly recessive (see Hutt & Lamoureux, 1940). Stubbe (1938, p. 79) gives a number of other examples showing that the same characters may be affected in much the same way by both dominant and recessive genes. In *Verbena* two identical chemical changes can be brought about in these two ways (Beale *et al.* 1940).

To what extent, then, can one assume that a dominant gene is producing a more advanced chemical product than its recessive allelomorph? The question is worth answering because it sometimes happens that the dominance relations of a given pair of allelomorphs are understood but it is not known which is the wild-type and which the mutant gene. From the above considerations, it is clear that one must first determine whether the organism is one in which mutants are nearly always recessive, like *Drosophila*, or one in which there is a high proportion of incompletely recessive mutations, like *Gallus*. If it belongs to the latter class, no deductions whatever can be made; but if to the former,

one is fairly safe in taking a recessive, even when it is not known what relationship it bears to the wild type, as producing a simpler substance or a less developed organ than a dominant. This is simply because a recessive gene is, most probably, also a mutant gene.

(c) *Gene interaction*

In physiological work, valuable information can be obtained from the interaction of various external factors such as temperature, light, etc. It is therefore reasonable to suppose that genetic interactions will also yield results from which deductions concerning synthetic processes can be made.

A common form of gene interaction, where two pairs of allelomorphs, say **A**, **a** and **B**, **b**, are involved, is epistasy, of which two types can be distinguished: (1) the phenotypes **aB** and **ab** may be indistinguishable, so that a 9 : 3 : 4 ratio is obtained in  $F_2$ , and (2) the phenotypes **AB** and **Ab** are indistinguishable, so that a 12 : 3 : 1 ratio is obtained in  $F_2$ . (Dominance of wild-type allelomorphs is assumed.)

Some examples of the first type have an obvious physiological explanation. Thus if one gene **A** controls the production of a pigment (e.g. an anthocyanin) and a second gene **B** the modification of that pigment (e.g. by changing the *pH* of the cell sap) it is obvious that **B** cannot show any effect until **A** has already done its work. **B** is therefore hypostatic. The important question is, however, can one deduce from the fact that a gene is hypostatic, that the reaction controlled by it occurs subsequent to that controlled by the epistatic gene? The *initial* processes brought about by the hypostatic gene may of course occur before the epistatic gene is acting; in the example given above, the gene **B** can alter the *pH* of the cell sap whether there is any pigment present or not. But the effect by which the gene is recognized as being hypostatic, namely, the change in colour, is the *final* result of the gene's activity. If one were to score the effect of **B** by measuring the *pH* directly, there would not be three but four distinguishable phenotypes, and therefore no case of epistasy. Accordingly, if there are two substances *X* and *Y*, and two genes **A** and **B**, as follows:

Genes	Substance
<b>ab</b> }	—
<b>aB</b> }	—
<b>Ab</b>	<i>X</i>
<b>AB</b>	<i>Y</i>

then it must be inferred that the gene **B** cannot bring about its reaction, i.e. the formation of substance *Y*, unless the gene **A** has already brought

about the formation of substance *X*. Hence, with this arrangement, the formation of *X* is an essential intermediate stage in the synthesis of *Y*. It is possible, however, that other genes are able to bring about the synthesis of *Y* by another method, as is discussed at the conclusion of this paper.

The second type of epistasy, that in which it is the wild-type allelomorph which prevents the segregation of a second gene from being observed, so that a 12 : 3 : 1 ratio is obtained in  $F_2$ , is more difficult to account for physiologically. (For example see pigment inheritance in *Lathyrus* and *Streptocarpus*, p. 208.) Here no important function can be attributed to the hypostatic gene (at least in regard to the particular character by which the epistasy is recognized), for whether it is present or absent in the wild-type the result is the same. Possibly the hypostatic gene is a relic from some previous time when the epistatic gene had not yet become established. According to this view, epistasy like dominance would be the result of selection, for when one gene is able to carry out the same function as another, and something else in addition, it is evidently advantageous for the first gene to become epistatic, so that mutations of the second to less viable types will be hidden.<sup>1</sup>

## II. APPLICATION TO EXISTING DATA ON ANTHOCYANINS

The inherited qualitative variations of the anthocyanin pigments are (1) variations in the number of hydroxyl (or methoxyl) groups at the 3' and 5' positions; i.e. the anthocyanins may belong to the delphinidin, cyanidin or pelargonidin classes (oxidation differences); (2) variations in the sugar residues at the 3 and 5 positions (glycosidal differences); (3) pH differences. Other variations, such as methylation or acetylation, cannot be considered because there are insufficient examples.

### (a) Variations in state of oxidation

In Table 1 are collected together all the species in which the wild type is known with tolerable certainty to contain a pigment belonging to one of the anthocyanidin types, and to have mutated at least once to one of the other types.

In those species where the colour variations are slight, and where the wild-type pigment was not already known, I have obtained material

<sup>1</sup> It is assumed that the recessive allelomorphs of the genes considered have no effect on the reactions controlled by the respective dominants. This assumption has been adopted merely for the sake of simplicity, since it is easier to speak of "the function of the gene *A*" rather than "the difference between the functions of *a* and *A*".

from the wild and tested the pigment. But, for the most part, descriptions or colour plates are adequate for the identification of the wild-type pigment when several varieties in cultivation have already been studied, or where there are not too many varieties.<sup>1</sup> For all the species given in Table 1 it

Table 1. *Direction of mutation from wild type to mutant*

Delphinidin → Cyanidin	Cyanidin → Delphinidin
<i>Ajuga reptans</i>	None
<i>Aster amellus</i> (1)	
<i>Clematis Viticella</i> (1)	
<i>Collinsia bicolor</i>	
<i>Gilia tricolor</i>	
<i>Lathyrus odoratus</i> (2)	
<i>Pisum sativum</i>	
<i>Prunella grandiflora</i> (2)	
<i>Phlox Drummondii</i>	
<i>Salvia Horminum</i>	
<i>Scilla non-scripta</i> (2)	
<i>Trifolium pratense</i> (4)	
Delphinidin → Pelargonidin	Pelargonidin → Delphinidin
<i>Campanula Medium</i>	<i>Anagallis arvensis</i> (2)
<i>Clarkia elegans</i> (2)	<i>Salvia splendens</i>
<i>Convolvulus bicolor</i>	
<i>Delphinium Ajacis</i> (1)	
<i>Hyacinthus orientalis</i> (2)	
<i>Hyssopus vulgaris</i>	
<i>Linaria alpina</i>	
<i>L. purpurea</i>	
<i>Primula sinensis</i> (2)	
<i>Veronica spicata</i>	
Cyanidin → Pelargonidin	Pelargonidin → Cyanidin
<i>Antirrhinum majus</i> (3)	<i>Papaver Rhoeas</i> (2), (5)
<i>Centaurea cyanus</i> (2)	
<i>Dianthus caryophyllus</i>	
<i>D. barbatus</i> (2)	
<i>Lathyrus odoratus</i> (2)	
<i>Matthiola incana</i> (2)	
<i>Verbascum phoeniceum</i> (4)	

(1) Several wild species might have been involved, but any possible ones can be assumed to contain the same wild-type pigment. Original chemical data.

(2) Chemical data given by Lawrence *et al.* (1939a).

(3) Chemical data given by Scott-Moncrieff (1936).

(4) Unpublished data of J. R. Price.

(5) *P. Rhoeas* has also "suppressors" of cyanidin, which can be considered as back mutations from cyanidin → pelargonidin (Scott-Moncrieff, 1936).

The remainder are based on original data.

is safe to assume that a mutation in the specified direction has taken place at least once. Back mutations, and mutations from allelomorphs unspecified as regards the wild type (e.g. the mutation from a pelargonidin to a cyanidin derivative in *Rosa polyantha* noted by Scott-Moncrieff, 1936) have been ignored.

<sup>1</sup> In this connexion I wish to thank Mr H. B. Parks of San Antonio, Texas, for sending me wild material of *Phlox Drummondii*.

In Table 2 are presented the species in which it is known which pigment form is dominant. A fair proportion of species occur in both Tables 1 and 2.

Table 2. *Dominance of pigment types*

Delphinidin dominant to cyanidin	Cyanidin dominant to delphinidin
<i>Callistemma chinensis</i> (1)	<i>Nemesia</i> (hybrids) (9)
<i>Lathyrus odoratus</i> (2)	
<i>Pisum sativum</i> (8)	
<i>Salvia Horminum</i> (8)	
<i>Streptocarpus</i> (garden hybrid) (3)	
<i>Trifolium pratense</i> (5)	
Delphinidin dominant to pelargonidin	Pelargonidin dominant to delphinidin
<i>Callistemma chinensis</i> (1)	<i>Anagallis arvensis</i> (7)
<i>Campanula Medium</i> (8)	<i>Verbena</i> (garden hybrid) (6)
<i>Clarkia elegans</i> (7)	
<i>Lathyrus odoratus</i> (2)	
<i>Linaria alpina</i> (8)	
<i>Pelargonium zonale</i> (2)	
<i>Primula sinensis</i> (2)	
<i>Salvia splendens</i> (4)	
<i>Streptocarpus</i> (garden hybrid) (3)	
<i>Verbena</i> (garden hybrid) (6)	
Cyanidin dominant to pelargonidin	Pelargonidin dominant to cyanidin
<i>Antirrhinum majus</i> (2)	<i>Papaver Rhoeas</i> (2)
<i>Cheiranthus Cheiri</i> (2)	
<i>Matthiola incana</i> (7)	
<i>Papaver Rhoeas</i> (2)	
<i>Pharbitis nil</i> (2)	
? <i>Zea mays</i> (2)	

Data collected from

- (1) Wit (1937).
- (2) Scott-Moncrieff (1936).
- (3) Lawrence *et al.* (1939b).
- (4) See text.
- (5) Price and Williams, unpublished.
- (6) Beale *et al.* (1940).
- (7) Chemical data from Lawrence *et al.* (1939b); genetical data summarized in Matsuura (1933).
- (8) Unpublished chemical data; genetical from Matsuura.
- (9) Mather (unpublished).

These tables illustrate two rules: (1) that mutation is predominantly from the more oxidized to the less oxidized<sup>1</sup> anthocyanins, and (2) that mutant types are recessive, whatever the chemical nature of the change produced by the mutation.

The relationship between delphinidin and cyanidin is strikingly regular; in twelve species there has been mutation from a delphinidin wild type to a cyanidin mutant, while no examples of mutation in a

<sup>1</sup> Delphinidin derivatives can conveniently be said to be more "oxidized" than cyanidin, and cyanidin than pelargonidin.

reverse direction are known. In six species (four being also included in the above twelve) delphinidin derivatives are dominant to cyanidin, and there is only one example of the opposite effect, viz. hybrids of *Nemesia strumosa* with *N. pulchella* and *N. versicolor* (Mather, unpublished). Therefore it seems clear that cyanidin derivatives are synthetically simpler than delphinidin.

The relationships between pelargonidin and the other two anthocyanins are somewhat less regular. Ten species are known to have mutated from a delphinidin wild type to pelargonidin, and seven from cyanidin to pelargonidin, while the numbers of the reverse processes are two and one respectively. In ten species the delphinidin type is dominant to pelargonidin and in two recessive, while in five species the cyanidin type is dominant to pelargonidin and in one recessive. Pelargonidin types are therefore probably at an earlier stage of development than either cyanidin or delphinidin, though this is not entirely significant because of the lower proportion of pelargonidin-containing wild forms. It would be desirable to examine more tropical species, where pelargonidin occurs more frequently in the wild, and to find out whether these also are more stable than the delphinidin or cyanidin species.

In considering the data in Tables 1 and 2 it is important to bear in mind that species which contain delphinidin and cyanidin derivatives in the wild occur with approximately equal frequencies (the former slightly predominating), while the pelargonidin type is less common, at least in temperate regions (Lawrence, Price, Robinson & Robinson, 1939). No exact percentages can be given because of the arbitrary selection of those species which have been tested.

According to Scott-Moncrieff (1936), more oxidized anthocyanins tend to be dominant to less oxidized. We now have a number of exceptions to this rule, viz. *Anagallis arvensis*, *Papaver Rhoeas*, *Verbena* (hybrid) and *Nemesia* (hybrid). In *Anagallis* and *Papaver* the direction of mutation from wild type to mutant was from the less oxidized to the more oxidized (i.e. the rare direction). In *Verbena* and *Nemesia*, the different anthocyanidin types were derived from the wild forms of different species. Regular dominance relations are therefore not to be expected in these hybrids.

These examples demonstrate the correlation of dominance with wild-type allelomorphs *irrespective* of the chemical substances produced. The only anomaly is *Salvia splendens*, which contains a pelargonidin derivative in the presumed wild form, and has given rise to a violet mutant containing a delphinidin derivative. The latter is apparently

dominant, judging by the appearance of the hybrid between the two forms (Beale, unpublished).<sup>1</sup>

So far, only single gene differences have been considered, and these serve for the determination of the first two "gene relations"—mutation and dominance—specified at the beginning of this paper. The third relation—epistasy—can only be investigated when there are more than two variants of a particular character; in the case of the anthocyanins, only when all three types—delphinidin, cyanidin and pelargonidin—occur in the same species. This happens in *Lathyrus odoratus*, *Streptocarpus*, and *Callistemma chinensis*.

In *Lathyrus* the wild form is known to contain a delphinidin derivative (Beale *et al.* 1939), and in *Streptocarpus* there are two wild species involved, *S. Dunnii* containing a cyanidin derivative and *S. Rexii* containing a delphinidin derivative (Lawrence *et al.* 1939*b*).

In both the same type of inheritance is found, the factorial constitutions being of the following type:

AB	}	delphinidin
Ab		
aB		cyanidin
ab		pelargonidin

i.e. **A** is epistatic to **B**. If the contention of Lawrence *et al.* (1939*a*), that cyanidin is the synthetically simplest form, is correct, it must be assumed that the oxidation process (to delphinidin) is able to override the reduction process (to pelargonidin), and it is difficult to see any selective advantage for this. But more examples of species in which all three pigments occur are required before one can draw any but tentative conclusions.

Wit (1937), in an extensive paper on the genetics of the China aster, *Callistemma chinensis*, postulates three multiple allelomorphs—**R**, **r'** and **r**—corresponding to the delphinidin, cyanidin and pelargonidin types respectively. This cannot be regarded as by any means certain, because (1) Wit, on p. 96, makes the statement "that flowers of the lilac (i.e. **r'**) group contain principally cyanidin glycoside is therefore not absolutely proved". I have tested some lilac coloured strains (obtained from commercial stocks) and have never found them to contain a pure cyanidin derivative, but always a mixture of delphinidin and pelargonidin with possibly some cyanidin derivative. (2) There is also some doubt about the allelomorphism of **R**, **r'** and **r**, as noted by Wit

<sup>1</sup> I should be very grateful for authentic wild material of *Salvia splendens* from Brazil.

on p. 99 of the cited paper. This is due to the fact that the results bearing on this question are not based entirely on controlled crosses but on the progeny of "rogues" (see Wit, 1937, Table 38).

In *Streptocarpus* it is noteworthy that one of the wild species—*S. Rexii*—has the constitution **Ab**, and the other—*S. Dunnii*—**aB** (adhering to the factorial scheme given above). To produce a pelargonidin type, it is only necessary to cross the two species and raise an  $F_2$ ; no new mutation is required. Presumably many of the species in Table 1, in which mutation has gone from delphinidin to pelargonidin, missing out cyanidin altogether, have a constitution similar to that of *S. Rexii*.

### (b) Glycosidal variations

The data on glycosidal differences are summarized in Table 3.

Table 3. *Direction of mutation and dominance of genes affecting glycosidal differences*

Diglycoside wild type → monoside mutant	Monoside wild type → diglycoside mutant
<i>Dianthus Caryophyllus</i> (1)	None
<i>Phlox Drummondii</i> (1)	
<i>Statice sinuata</i> (1)	
Diglycoside dominant to monoside	Monoside dominant to diglycoside
<i>Callistemma chinensis</i> (2)	<i>Verbena</i> (garden hybrid) (3)
<i>Verbena</i> (garden hybrid) (3)	

Data from (1) original; (2) Wit (1937); (3) Beale *et al.* (1940).

According to Lawrence *et al.* (1939*a*), monosides occur less frequently in the wild than diglycosides. It is therefore premature to attach any significance to the prevalence of changes in the direction wild-type (or dominant) diglycoside → mutant (or recessive) monoside until further data are obtained, though so far the results are consistent.

### (c) pH differences

Table 4 shows the species in which genetic pH differences have been recorded. All seven of them show the same behaviour: the more alkaline

Table 4. *Genetic data on pH differences*

Acid dominant to alkaline	Alkaline dominant to acid
<i>Lathyrus odoratus</i> (1)	None
<i>Papaver Rhoeas</i> (2)	
<i>Primula acaulis</i> (2)	
<i>P. sinensis</i> (2)	
<i>Trifolium pratense</i> (3)	
<i>Tropaeolum majus</i> (3)	
<i>Verbena</i> (garden hybrid) (4)	

Data from (1) Beale *et al.* (1939); (2) Scott-Moncrieff (1936); (3) Price, unpublished; (4) Beale *et al.* (1940). For direction of mutation, see text.



cell-sap is always recessive to the more acid, and in six out of the seven (i.e. all except *Primula acaulis*) the direction of mutation is known to be acid  $\rightarrow$  alkaline.

An additional point of interest is that in *Primula sinensis* it is only the petal-lobes which show any variation in pH; other parts of the plant are like the *alkaline* type of petal cells (Scott-Moncrieff, 1936). Thus mutation results in a raising of the abnormally low pH of the petals to that of the rest of the plant.

Assuming that the above seven species form a random sample, the inference is that natural selection has for some reason favoured the production of an exceptionally acid sap in the petals. This may be related to the fact that anthocyanins are less stable in alkaline than in acid solution, at least *in vitro*.

### III. CONCLUSION

The foregoing data show that homologous variations affecting anthocyanins in different species are inherited according to a regular system. Thus, the delphinidin-cyanidin difference is always found to be controlled by a pair of allelomorphs, whose wild-type member is associated with a delphinidin derivative and whose mutant member is associated with a cyanidin derivative. Similarly, where there are sugar differences, it is the diglycoside which is found in the wild type, and the monoside in the mutant; and where there are pH differences, mutation from a wild-type allelomorph has always led to the formation of a more alkaline cell sap, and not a more acid one. A consequence of this regularity in direction of mutation is that delphinidin types are dominant over cyanidin, diglycosides over monosides, and acid over alkaline cell sap. Dominance is only irregular when interspecific hybrids are involved, e.g. in the *Nemesia* hybrids where cyanidin is dominant over delphinidin, and in the *Verbena* hybrids where monoside may be dominant over diglycoside. This is only to be expected if the mechanism of selection for dominance is different in different species.

Reasons for the consistency with which wild types containing a given pigment mutate in the same direction are suggested earlier in this paper. It remains here to consider the exceptions. Table 1 shows that there are two species which contain a pelargonidin derivative in the wild type and which have produced mutants with delphinidin; and one other species which contains pelargonidin and has produced a cyanidin mutant. There are seventeen species in which mutation has been in the reverse direction, not counting six others in which the wild type is not known, but can be

deduced from the dominance. Mutation away from the wild type is therefore preponderantly in the direction delphinidin or cyanidin  $\rightarrow$  pelargonidin, even allowing for the lower proportion of wild pelargonidin-containing species. But there are the three exceptions mentioned above.

These might be accounted for by assuming that the difference between pelargonidin and the others is so slight that mutation can go equally easily in either direction. This is not a satisfactory explanation because cyanidin and delphinidin are also very similar, but the production of a delphinidin mutant from a cyanidin wild type has never been known to occur.

Hitherto it has been assumed that the details of the synthesis of the anthocyanins are the same in different species. There are however genetical considerations that indicate that this is not always true. First, genes at different loci rarely, if ever, produce absolutely identical effects. This is demonstrated by the well-known fact that if two, say, chlorophyll-deficient plants, each containing a different recessive gene, are crossed, the hybrid will have normal green leaves. (In practice such behaviour is taken as proof of non-allelomorphism.) The dominant allelomorphs of the two chlorophyll-deficient genes (or "complementary factors") must therefore have different functions. Genuine examples of "duplicate factors" (i.e. two genes at different loci with identical functions, and therefore yielding a 15 : 1  $F_2$  ratio) probably do not occur except in polyploids.

Secondly, the reactions controlled by a given gene do not remain always the same, but may be taken over, as mutations occur, by other genes. Muller (1939) has discussed at length this "transference of function" of genes. Even though the appearance of an organism in regard to a particular character remains constant, the genetic determiners of that character may have changed. Convincing demonstration of this is given by Harland (1936), who shows that different species of *Gossypium* may have apparently similar characters, controlled by widely differing gene systems. This shift of control does not seem always to occur with genes which produce striking effects, for in *Drosophila* it is known that different species (*D. melanogaster*, *D. affinis*, *D. pseudoobscura*) have their homologous genes in roughly corresponding linkage groups (Sturtevant, 1940). These genes must have retained much the same function from a time before the different species diverged from their common ancestor.

Taking together the two facts that genes at different loci act differently, and that in course of time a given character may come to be controlled

by a different set of genes, it is reasonable to infer that a substance is not always synthesized by the same chain of processes in different species, though certain fundamental steps would of course always be necessary. Such variation in the synthetic processes would explain the apparent anomalies of some of the genes controlling the difference between pelargonidin and the other two anthocyanidins, but there is no critical evidence on this point.

The present state of our knowledge does not admit a satisfactory answer to these questions. Either more details must be obtained by purely physiological methods, and collated with the genetical data, or a more rigorous examination of such little understood phenomena as mutation frequency must first be made so that their physiological significance can be more definitely established. However, it is hoped that the present analysis provides a framework into which the results of future investigations could be fitted.

#### IV. SUMMARY

The genetic phenomena of mutation, dominance and epistasy are analysed to discover if they may be used as indicators of the direction of synthetic processes. From general considerations the following conclusions are drawn:

(1) Mutation away from a standard wild type usually results in the formation of simpler substances, i.e. those requiring a smaller number of stages for their synthesis. Hence, where there is a qualitative difference brought about by a gene substitution, the substance associated with the mutant gene is likely to be an intermediate product in the synthesis of the substance associated with the wild-type gene.

(2) Dominance cannot be used as an indicator of synthetic processes in those organisms which have a proportion of non-recessive mutants, since there is no sign that dominant mutants differ from recessive mutants in any developmental way. But dominance of the wild type in most organisms is fairly complete and therefore direction of mutation, when not already known, can be inferred.

(3) Epistasy of the type which gives a 9 : 3 : 4  $F_2$  ratio is concluded to be an indication that a reaction brought about by an epistatic gene is an essential precursor of a reaction brought about by the corresponding hypostatic gene.

These conclusions are applied to variations affecting anthocyanins. It is deduced that cyanidin derivatives are synthetically simpler than delphinidin, but the relation of pelargonidin derivatives to the other

anthocyanins is not clear. Monosides are apparently simpler than diglycosides, though more data are required on this type of substitution. All the known mutations affecting *pH* of the petal cell sap are in the direction acid  $\rightarrow$  alkaline. Natural selection has apparently favoured the production of a more acid sap in the petals than in the plant in general.

The anthocyanin data confirm that: (1) Changes in the wild-type phenotype do not occur at random, but preponderantly in one direction, from the complex to the simple, and (2) That dominance is associated with wild-type allelomorphs *irrespective* of the nature of the chemical changes produced by a gene substitution.

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## WAVED—A NEW COAT TYPE IN RABBITS

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(With Plate 15)

### *1st mating.*

IN the autumn of 1932 a number of fine-coated Rex rabbits were purchased from a breeder, the majority of these rabbits being brown (Havana-Rex). As they were primarily required for studies relating to the possibilities of producing a sound strain of exhibition Rexes these rabbits were well up to show standard. Whilst the does were all normal plush-coated Rexes of exceedingly fine texture, two of the brown bucks had a slight waviness in their coat, especially on the back. This was not very marked until after a moult the following summer when the waviness was seen to be distributed throughout the coat (Pl. 15, fig. 1). None of the original does showed this character, nor in later tests did any of the other original rabbits appear to carry it. A number of litters were produced sired by one or other of these two bucks mated to normal-coated Rex does. These produced 23 youngsters, none of which could be classed as markedly waved, although three of them showed a very slight tendency to a waved condition on the back at some time or other in their early life. This was, however, simply due to the normal process of moult, and after the age of one year, no waves were perceptible in any of the animals. They were therefore classified as normal-coated Rexes.

### *2nd mating.*

An  $F_2$  generation consisting of 22 individuals was bred, of which 17 were classed as normal Rexes and 5 as waved, a very close approximation to the Mendelian expectation of 16.5 : 5.5, if the waved condition is a simple autosomal recessive character.  $F_1$  does mated to their wavy sire produced three litters including 8 normal Rexes and 7 waved Rexes. Four litters of waved  $\times$  waved produced 17 waved and no normal youngsters. From these preliminary crosses it could therefore be safely concluded that the waved condition of the coat is a simple recessive to normal coat as it appears in the Rex. In these and in all subsequent matings it was not found possible to distinguish between normals heterozygous for the waved condition and those which were homozygous.

*3rd mating.*

A litter bred from brown waved  $\times$  albino shorthair produced all shorthairs. These bred *inter se* gave 28 shorthairs and 6 waved Rexes, all of the fine-coated type. In this and most subsequent matings it is interesting to note that the ratio of normals to Rexes is wider than the Mendelian expectation, but this supports previous figures where the production of Rexes is less than anticipated. None of the shorthaired individuals appeared to vary from the normal. It was, however, remarkable that no normal Rexes had been produced in this cross. Two possible explanations were suggested, either that these three characters of normal shorthair, normal Rex, and waved formed an allelomorphic series, or that the original shorthair was in fact a "disguised waved", this character not being able to manifest itself unless combined with Rex.

*4th mating.*

In order to investigate further these possibilities, an entirely unrelated shorthair (blue) was crossed to a brown waved buck. The  $F_1$  consisted of four normal shorthairs, whilst the  $F_2$  gave 58 shorthairs and 15 Rexes of which only three were waved. It appeared, therefore, that the second explanation was correct and that the shorthair used in the original cross had, in fact, been a disguised waved.

In the course of correspondence with a breeder who owned a strain of similar waved Rexes, the suggestion was made that this characteristic might in some way be related to the Angora. This postulation received some support by the fact that the albino shorthaired doe used in the first shorthair and waved cross had as her grandsire an Angora, and although she was not herself heterozygous for the Angora character, it had been shown that she was, in fact, a disguised waved.

*5th and 6th matings.*

To investigate this matter further, waved Rex were twice crossed to an Angora Rex doe, and all the resulting nine youngsters proved to be waved. A further cross was made between an exhibition type normal Angora and a waved Rex. The resulting litter of five consisted of four shorthairs and one Angora, showing that in this case the waved Rex used had been heterozygous for Angora. The shorthairs from this cross when bred *inter se* produced an  $F_2$  of 18, of which ten were shorthairs, three were Angoras, and five were waved Rexes. Unless it died in the nest at too early an age for identification, the expected Angora Rex did not appear, but the numbers were small. It should be noted that no

normal Rexes were produced, which shows that the Angora used in the experiment must again have been a disguised waved.

*7th mating.*

A waved buck was mated to an exceedingly coarse coated, French type Angora doe. Owing to unfortunate circumstances that season, however, an  $F_2$  of only ten individuals was secured and of these only one Rex was reared. This proved to be a non-waved, which suggests that the waved characteristic is *not* carried by all Angoras, but may be confined to the finest exhibition British type of that variety.

Since three distinct strains of exhibition Angoras were used in these waved rabbit experiments, it seems probable that many if not all of these Angoras bear the waved characteristic in their genetical constitution. The Castorrex when first imported into this country was very generally mated to existing fur breeds in order to produce new Rex colours, and as these fur breeds had already in many instances been freely crossed to Angoras in order to improve the fur properties, it is not surprising that "disguised waveds" were used with the result that the waved coat made its appearance in several different strains of Rexes.

*1st and 8th matings.*

From the earliest matings one unexpected point appeared, the waved condition generally, but not always, appeared to be confined to the brown coat. In the first test of waved Rex  $\times$  normal Rex the latter does were homozygous black Rexes, the  $F_1$  all being black. In the  $F_2$ , however, all the 5 waved Rexes were brown, and the 17 normals were 15 blacks and 2 browns. In the original backcross, 6 brown waved Rexes and 1 black waved Rex were produced to 5 black and 3 brown normal Rexes, whilst one litter of black (heterozygous for brown) waved  $\times$  brown waved, produced 2 black waved and 3 brown waved.

*3rd mating.*

An albino doe which was known genetically to be a homozygous black was used as the normal shorthair in the first of those crosses whilst the waved Rex buck was brown. The  $F_1$  were all blacks, and the 6 waved Rexes of the  $F_2$  were 4 brown and 2 black. The  $F_2$  shorthairs were 18 blacks, 8 albino and 2 browns. Whilst the waved condition did not appear to be entirely confined to the brown colour it seemed to appear more readily in it than in any other, and the possibility of linkage could not be overlooked.



*4th mating.*

In the second cross of normal shorthair to waved, the latter was brown whilst the shorthair was blue. As was expected the four  $F_1$  rabbits were all blacks, and the  $F_2$  of 73 animals were classified as follows:

	Black	Brown	Blue	Lilac	Actual total	Expectation
Shorthair	31	12	12	3	58	54.8
Normal Rex	6	3	2	1	12	13.7
Waved	2	0	0	1	3	4.6
Actual totals	39	15	14	5	73	
Expectation	41.1	13.7	13.7	4.6		

*9th mating.*

In order to study further the possibility of linkage between either brown or blue and the waved condition, a brown waved buck was mated to a blue Rex doe, which was known not to be heterozygous for the waved factor. The resulting  $F_1$ , none of which were waved, were interbred and produced 72 youngsters, of these 21 were waved, and 51 normal Rexes, classified as follows:

	Black	Blue	Brown	Lilac	Total	Expectation
Normal Rexes	27	11	9	4	51	54
Expectation	30.4	10.1	10.1	3.4	54	—
Waved Rexes	13	2	4	2	21	18
Expectation	10.1	3.4	3.4	1.1	18	—
Totals	40	13	13	6	72	
Expectation	40.5	13.5	13.5	4.5		

This approximation to expectation is sufficiently close to show that when larger numbers of records were available, no evidence was forthcoming of possible linkage between the waved condition and either brown or blue colour. As brown has been shown to be linked with albinism, it is unlikely that the waved condition is in any way associated with albinism.

In a large proportion of the waved Rexes from the earlier matings the first moult, which begins when the rabbit is about 6 weeks old, appeared to be exceedingly rapid, sometimes being completed in as little as 15 days. In these cases the rabbit was generally absolutely naked for some days and needed especial care in housing and bedding to keep it sufficiently warm. Moults in later life appeared to follow the normal course.

The age at which the waved specimens can be distinguished from the normal varies. Generally, waved youngsters bred from waved parents can be distinguished as soon as their coat shows, at about 8–12 days. The majority of these were found in the early tests to have the complete (“naked”) moult, but later on in many matings youngsters, distinguished at an early age as waved by a “matt-like” appearance of their coats,

did not have the naked moult. In other cases, and especially those bred from crosses in which one or both parents were normal Rexes heterozygous for the waved condition, the youngsters may not be distinguishable from normals until 4 months or even older. The wave in all cases becomes more marked after each successive moult, reaching the maximum development at the age of 18 months or 2 years. By selection an early development of the wave can be fixed in a strain.

Waviness in the coat appears to be strictly confined to the finest coated exhibition types. It does not appear in the German type of Rex, nor in the coarser types of Gillet Rex, and experimental crosses have not been successful in producing a waved coarse-type Rex, but it would seem that they may be in some cases disguised waveds.

The losses in the waved Rexes have not been outstanding, but have been slightly above those of normal rabbits. In earlier experiments a number were lost during the first naked moult, but later litters in which these occurred were saved through methods of improved husbandry.

Whilst waved Rexes are not common, at least three breeders are in possession of types similar to those first bred at the Institute. It appears that data drawn from these studs would produce very similar conclusions to those stated above. An exception, however, is reported in one stud where the proportion of waved Rexes from normal  $\times$  waved Rex is approximately 3 : 1. This is higher than would be expected, even allowing for the majority of the normal Rexes being heterozygous for this type of coat. Possibly some of the litters recorded as from normals are really from young waveds which were late in developing their curl, or alternately from harsher coated animals which were actually masked waves. Recently stock has been secured of this waved strain and at the Institute the behaviour of its inheritance has been identical to that of the original stock reported upon in this paper.

The amount of wave in the coat varies considerably and appears without doubt to be affected by modifying factors. One breeder who has concentrated on the type has, by means of careful selection, produced a type that is exceedingly well waved, the coat being almost a mass of curls very similar to astrakan. This is now recognized as a variety, the *Astrex* (Pl. 15, fig. 2).

At the other extreme, some exhibition Rexes show a slight wave which is obviously due to the character, and is not desirable in the ordinary Rex. In view of the conclusions drawn in this paper, it should be a fairly simple matter for breeders either to perpetuate or to eliminate this character from their stock.

Nothing remarkable was noted in the sex-ratios of the rabbits produced under this experiment, but the numbers in any case were insufficient. Reciprocal crosses had similar results, and no evidence of sex-linkage was found.

This character of wave in rabbits appears to be similar to that reported by Crew (1933) in mice in that it is a simple autosomal monogenic recessive. Unlike the mutation in the mouse, the waved coat in the rabbit appears to become more marked with increasing age. This is similar to the dominant character shown in the curly-coated rat exhibited by Dr Helen King at the Sixth International Congress of Genetics at Ithaca in 1932.

#### SUMMARY

1. A waved coat condition in rabbits is described.
2. Evidence is produced to show that this character is a simple autosomal monogenic recessive.
3. No evidence of linkage with either blue or brown coat colour was found.
4. The character can only be manifested in the fine coated Rex type of rabbit, but it is commonly found in a masked form in other types, especially in exhibition Angoras, and in fur breeds descended from them.
5. The extent of the wave appears to be controlled by modifying factors.
6. In waved youngsters the moult is frequently exceedingly rapid, sometimes producing a naked condition.
7. The type has been discovered in several different strains of Rexes, but in all cases investigated the mode of inheritance has been similar.
8. The waved character is compared with somewhat similar mutations in the mouse and rat.

The author desires to acknowledge with gratitude helpful suggestions received during the course of this study from Mr M. S. Pease of Cambridge and Mr E. C. Richardson of West Byfleet, who have also read the MS. of the paper. He also wishes to express his appreciation to the breeders of similar stocks who have placed their records at his disposal and especially to Mrs A. de Ville Mather for permission to reproduce the illustration of her Astrex.

*Summary of matings*

1st F	Waved Rex (brown)	×	Normal Rex (black)		Backcross
	23 Normal Rex (black)	×	Waved Rex (brown)		
	17 Normal Rex (15 black and 2 brown)		5 waved Rex (all brown)	8 normal Rex (5 black 3 brown)	7 waved Rex (6 brown 1 black)
2nd			Waved Rex × Waved Rex 17 waved Rex		
3rd			Brown waved Rex × Albino shorthair (black) (disguised waved) All shorthairs (black) (disguised waved)		
	28 shorthairs (2 brown, 8 albino, 18 black) (disguised waved)		6 waved Rexes (4 brown, 2 black)		
4th			Brown waved Rex × Blue shorthair 4 black shorthair		
	58 shorthairs (including disguised waved)		12 normal Rex	3 waved Rexes	
	31 black 12 brown 12 blue 3 lilac		6 black 3 brown 2 blue 1 lilac	2 black 1 lilac	
5th			Waved Rex × Angora Rex (disguised waved) 9 waved Rexes		
6th			Waved Rex × Exhibition Angora (disguised waved) 4 shorthairs and 1 Angora (disguised waved)		
	10 shorthairs (disguised waved)		3 Angoras (disguised waved)	5 waved Rexes	0 Angora Rex
7th			Waved Rex × French Angora Shorthairs		
	9 shorthairs and Angoras		1 Rex	0 waved Rexes	
8th			Black waved Rex × Brown waved Rex (Het. brown) 5 waved Rexes (2 black 3 brown)		
9th			Brown waved Rex × Blue Rex Black Rexes		
	51 normal Rexes (27 black, 9 brown, 11 blue, 4 lilac)		21 waved Rexes (13 black, 4 brown, 2 blue, 2 lilac)		

## REFERENCE

CREW, F. A. E. (1933). 'Waved', an autosomal recessive coat form character in the mouse. *J. Genet.* **27**, 95-6.

## EXPLANATION OF PLATE 15

Fig. 1. Waved Rex pelt, from the Institute's stock.

Fig. 2. Astrex rabbit, showing effect of a concentration of modifiers.

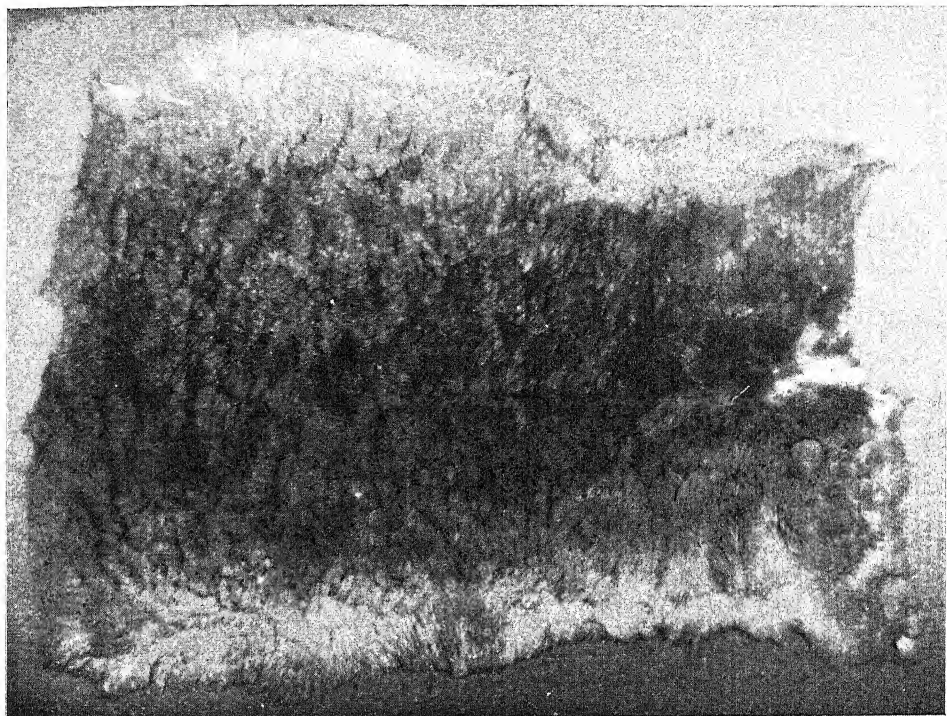
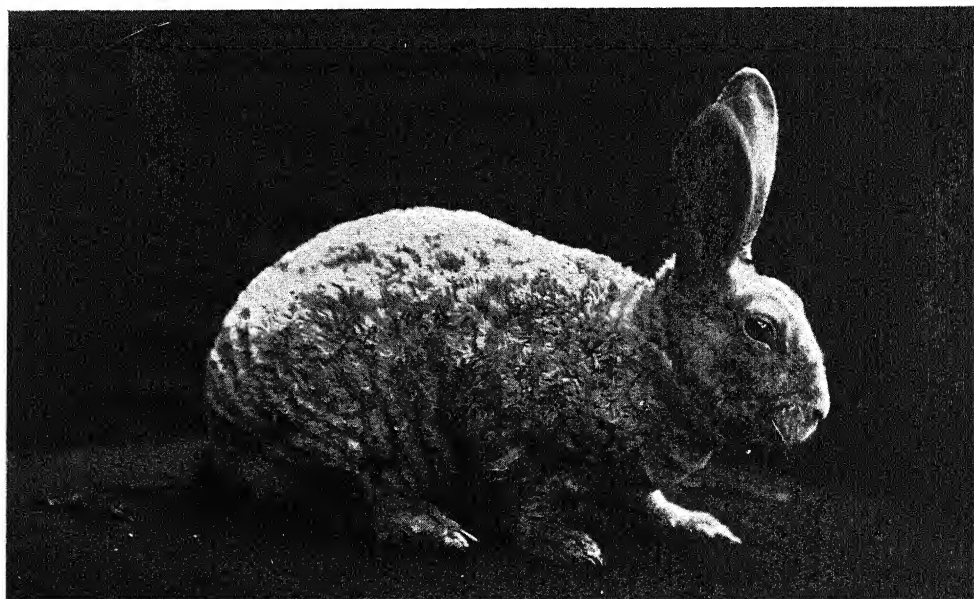


Fig. 1.





# CHROMOSOME BEHAVIOUR IN *UVULARIA*

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(With Plates 16 and 17 and Thirty-five Text-figures)

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## I. THE CENTROMERE AT MITOSIS

### 1. THE SOMATIC CHROMOSOMES OF *UVULARIA*

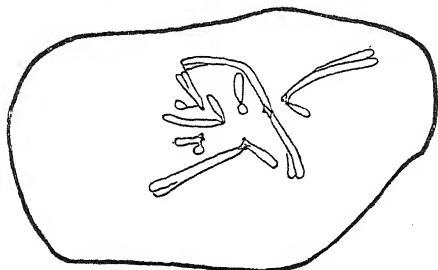
THE genus *Uvularia* is remarkable in that all the seven haploid chromosomes can be distinguished from one another. In this paper I shall give an account of differences in behaviour of the chromosomes of *Uvularia* and shall attempt to correlate these differences with the characteristics—length, position of the centromere, etc.—of the chromosomes concerned.

The plants used belonged to the *perfoliata-grandiflora* group and are all referred to as *U. perfoliata* in the text. No constant cytological differences were noted between the different clones in cultivation at



Merton. It is doubtful whether *perfoliata* and *grandiflora* can be separated (but cf. Anderson & Whitaker, 1934).

The somatic chromosomes were studied in root tips and pollen-grain smears (2BE-gentian violet). The two types of division are illustrated in Text-figs. 1 and 2. The chromosomes are very similar to those found by



Text-fig. 1.

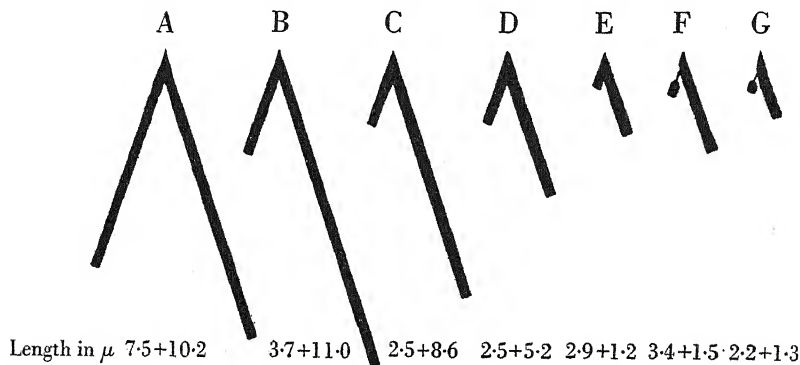
Text-fig. 1. Metaphase in pollen grain. Note the polarized centromeres.  $\times 1200$ .



Text-fig. 2.

Text-fig. 2. Metaphase in root tip.  $\times 1200$ .

Belling in *U. grandiflora*. The lengths of the arms appear to differ slightly in his and my material, but the differences are probably not significant. The chromosomes will be designated *A*, *B*, ..., *G*, in descending order of length. The lengths of the chromosomes and of their arms are given in the diagram (Text-fig. 3). The measurements were taken from pollen-grain metaphases and represent the mean of four or five observations.

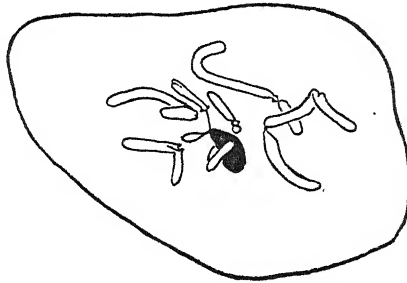


Text-fig. 3. Diagram of the somatic chromosomes of *Uvularia perfoliata*. The lengths of the two arms (in microns) are given below each chromosome.

Each chromosome has a single centric constriction which separates two arms of different lengths. In chromosomes *F* and *G* the constrictions are very well defined, the centromeres nearly coinciding with nucleolar organizers in both chromosomes. At prophase these chromosomes remain

attached to the nucleolus (Text-fig. 4). These are the only two nucleolar organizers in the haploid complement.

There is no evidence of secondary pairing in mitosis. In root-tip divisions the smaller chromosomes tend to lie on the inside of the plate, but the arrangement is very variable. In the resting nucleus, which is of the "solid" type (Manton, 1935), the chromosomes retain the orientation derived from the preceding telophase. At early prophase they appear



Text-fig. 4. Late prophase of pollen grain. The two nucleolar chromosomes, *F* and *G*, are still attached to the nucleolus. Note the already divided centromeres.  $\times 1200$ .

with the centromeres directed to one pole, the arms lying more or less parallel curving round the nucleus. The ends of the longer arms usually form a group at the pole of the nucleus opposite to the centromere.

## 2. THE BEHAVIOUR OF THE CENTROMERE

The contradiction between the movement of the chromosomes away from the poles of the spindle on to a plate and their later movement away from the plate towards the poles presents us with one of the important problems of cell mechanics. It is represented in conventional terminology as the change from metaphase to anaphase. I have described some of its physical conditions in the living cell (1939). I now propose to describe certain evidence of its mechanical character from fixed material.

The normal appearance of the centromere at metaphase in *Uvularia* pollen grains is shown in Pl. 16, fig. 1. In each chromosome it is orientated separately and is conspicuously bipolar. The chromosome thread passing through the centromeres (carrying centromeres; Darlington, 1939) is already divided at metaphase. In faded preparations the daughter centromeres may remain as two deeply stained granules one above the other, just as the two co-orientated centromeres in a meiotic bivalent sometimes hold the stain longer than does the chromosome body. In fact the configurations shown in Pl. 16, fig. 1, are strikingly like those in bivalents

with two chiasmata close on either side of the centromere. The distance between the centromeres apparently increases gradually during the metaphase, this cell being at late metaphase.

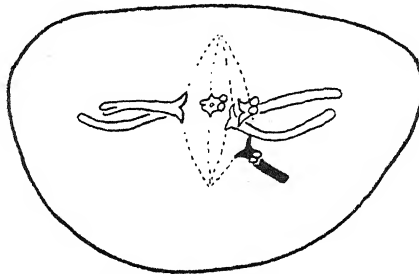
At prometaphase, when the chromosomes are coming on to the plate, the centrogenes are already divided. Text-fig. 4 shows such a stage, with the nucleolus still persisting. The two centrogenes in each chromosome are very close together. The time of division is probably at the end of prophase, since at late prophase the centric constriction can often be recognized as an unstained gap towards which the two chromatids converge. Metaphase orientation at mitosis in *Uvularia* is consequent on division of the centrogenes.

In other organisms the sequence of events is slightly different. For example, in *Tradescantia* or *Paris* pollen grains the stage shown in Pl. 16, fig. 1, rarely occurs. Less than 10 % of metaphases show the bipolar centromeres projecting up and down from the body of the chromatid, whereas in *Uvularia* over 90 % of the metaphase plates show this type of configuration. It appears as if the repulsion cycle of the centromere is more advanced relative to the onset of anaphase in *Uvularia* than in other organisms.

The onset of anaphase in certain grasshopper hybrids has been shown by Klingstedt (1939) to be conditioned both by the lapse of chromatid attraction and by the development of a repulsion within the mitotic centromere or between the meiotic centromeres. In his hybrids, the centromeres may start moving before the lapse of attraction, and this may lead to breakage of the chromosomes. In the parental species, the two events are exactly synchronized. The difference between centromere behaviour in *Paris* and *Uvularia* pollen grains is a less extreme example of the same type of variation. In *Uvularia*, to a less extent than in the hybrid grasshoppers, the lapse of chromatid attraction is delayed with respect to time of centromere division, while in *Paris*, etc., these two events are more nearly synchronized, so that the centrogenes pull themselves out of the body of the chromatid either rarely or for a very short time. That this is the correct explanation is made more probable by the behaviour of the centromere in *Uvularia* after the anaphase movement has begun. It does not project from the body of the chromatid. Apparently the chromatid body by virtue of its elasticity has caught up with its centromere.

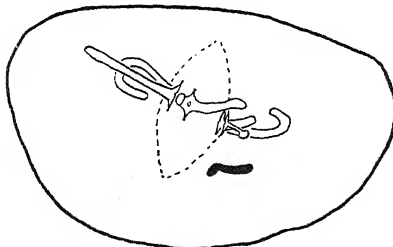
## 3. NON-CONGRESSION AND NON-ORIENTATION

The orientation of the centromeres on the metaphase plate almost always takes place as described in the last section. Rarely, chromosomes may fail to come on to the plate. Text-fig. 5 shows a pollen grain at metaphase with the *D* chromosome off the plate. It has, however, managed to orientate on one of the spindle arcs and the centromere is bipolar as usual. Presumably the anaphase would end normally in spite of lagging on one side.

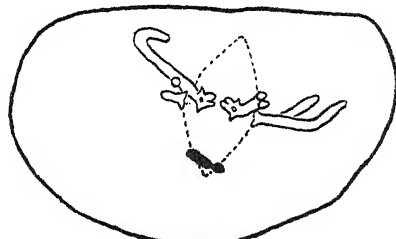


Text-fig. 5. Pollen-grain metaphase with *D* chromosome off the plate but with the centromere orientated on the spindle.  $\times 1200$ .

Text-figs. 6 and 7 show another type of non-congression. In these cells a chromosome has been left completely off the spindle. The centromere is unpolarized in the unorientated chromosome. Such non-orientation will probably lead to the loss of the chromosome concerned or perhaps to the formation of an  $(x-1)$  and an  $(x+1)$  nucleus. The



Text-fig. 6.



Text-fig. 7.

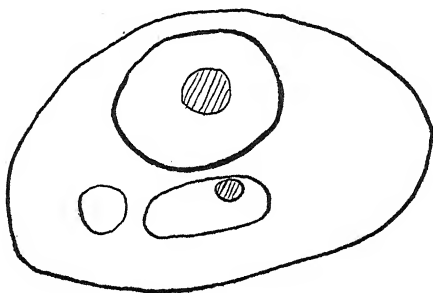
Text-figs. 6, 7. Pollen-grain metaphase with the *E* chromosome off the spindle. Its centromere does not show the bipolarity of the orientated centromeres.  $\times 1200$ .

binucleate pollen grains occasionally show a micronucleus (Text-fig. 8) which may have been derived in this way.

This behaviour is similar to that of small fragment chromosomes which may divide asynchronously at anaphase in certain species, e.g.

*Tulipa galatica* (Upcott, unpublished, illustrated by Darlington, 1937). Sometimes they do not appear to orientate normally. Probably the variations in number of such fragment chromosomes within the same plant, which have been observed by Langlet (1927) in *Ranunculus acris* and Darlington (1937*a*) in *Tradescantia*, arise in this way. Non-orientation has also been observed in pollen grains of *Tulipa* (Upcott, 1939*a*) and *Fritillaria* (Darlington, 1936, 1940*a*).

There seem to be two reasons for non-orientation in these cases. Sometimes spindle development is abnormal, e.g. in the pollen grains of *Tulipa*. In the other case the chromosomes are fragments recently formed by structural change. Their centromeres appear ill-adapted to the requirements of mitosis. Similar non-orientation occurs in the new telocentric chromosomes which arise by misdivision of the centromere



Text-fig. 8. Micronucleus in pollen grain after first pollen-grain mitosis.  $\times 1200$ .

in *Fritillaria latifolia* 3*x* (Darlington, 1940*a*), where the centromere is fragmented and reduced in size.

There is some evidence that similar variation in efficiency may occur between the centromeres in *Uvularia*. Only three cases of non-orientation in the pollen grains have been observed in over 200 metaphases, and in each case the *E* chromosome was concerned. There is only a 1 in 49 chance that non-orientation would affect the same chromosome three times if the probability of its occurrence is the same for all seven chromosomes. Further, we might expect, a priori, that the largest chromosomes would suffer most. Their centromeres have more work to do. The fact that at mitosis a short chromosome should be most liable to non-orientation may mean that its centromere is not so efficient as those in the other chromosomes; in other words, that genetic variation occurs among centromeres as among chromosomes.

## II. THE SEQUENCE OF PAIRING AT MEIOSIS

## 1. INTRODUCTION

Chromosome pairing at pachytene is a first condition for the formation of chiasmata. If pairing is limited to certain regions of the chromosomes, chiasmata are similarly limited or localized. Thus the proximal localization of chiasmata in species of *Fritillaria* follows a proximally localized pachytene pairing. The difference between these species, e.g. *F. Meleagris*, and others with chiasmata distributed all along the chromosomes, e.g. *F. imperialis*, is considered by Darlington (1936, 1940*b*) to be caused by the action of a *time limit* in pairing. Pairing begins proximally in both types, but does not proceed to completion in those with localized chiasmata. The latter have a shorter time in which to pair, or they are slower in starting.

The present paper gives an account of experiments which were designed to limit the pachytene pairing artificially in a plant where in the natural condition the chiasmata are distributed over the whole length of the chromosomes. For this purpose heat shocks, which have been shown to reduce chiasma frequency in several plants (*Tradescantia*, Sax, 1937; *Trillium*, Matsuura, 1937) were used.

*Uvularia perfoliata* was selected as experimental material because the wide variation in chromosome type within the complement makes it possible to discover the different reactions of chromosomes of different sizes, and also because it was found that considerable variation in the chiasma frequency occurs under natural conditions.

## 2. MATERIAL AND METHODS

Plants of *U. perfoliata* growing in pots were subjected to various temperature shocks when the anthers were at early prophase of meiosis, i.e. leptotene-zygotene. The most successful treatments were 2 days at 30° C. and 4 days at 30° C., the treatments beginning about 10 days before meiosis. They cause a great reduction in chiasma frequency. Treatment at a later stage does not have nearly so great an effect on chiasma formation (cf. Barber, 1940, on *Fritillaria*), presumably because when pairing is complete the conditions determining crossing-over are laid down and are not alterable by this treatment.

Fixations were made immediately after treatment and a few days later. Both 2BE-gentian violet and acetocarmine smears were used.

Control fixations from plants growing under "normal" conditions, i.e. in cold frames in March, were also made. Fixations made in different

years show fairly large variations in chiasma frequency, presumably owing as we shall see to naturally occurring temperature changes.

### 3. MEIOSIS

Meiosis in *U. grandiflora*, a species very similar to *U. perfoliata*, has been described by Belling (1925, 1926). He was able to recognize the seven different bivalents, but in the present material it is difficult to distinguish with certainty between bivalents *B* and *C*, the two long sub-terminals. There is also very little difference between the short bivalents *E*, *F* and *G*. In the tables these bivalents are grouped.

No exact analysis of chromosome pairing is possible at zygotene. Pachytene and diplotene nuclei show a tangled mass of threads with a nucleolus fitting like a cap over one side of the nucleus. Earlier the nucleolus is a spherical body situated centrally in the nucleus. There is no movement of chiasmata after their formation, since terminalization coefficients at full metaphase are small (see Tables 1-4). With decrease in chiasma frequency by treatment the terminalization coefficients do not become greater, showing that the decrease is not brought about by increased terminalization.

### 4. CHIASMA FREQUENCY

Chiasma frequencies for the separate bivalents and bivalent arms which can be recognized are given in Tables 1-4 and graphically in Text-fig. 13. The data are from four plants which received different treatments. The frequencies given in Tables 1 and 2 are from plants which received "normal" treatment. The difference in the chiasma frequency presumably represents the effect of the different environmental conditions, as the fixations were made in different years (1937, 1938) from the same plant. Belling (1925, 1926) obtained a similar reduction in *U. grandiflora*. Some of his plants were subjected to a late frost just before the meiotic divisions took place. The mean chiasma frequency of the cells he figures is only about 13. The reduction is not so extreme in the above plants—the nuclear means are 23.5 and 18.3 respectively.

The data in Tables 3 and 4 are from the plants which received the shorter and longer heat shocks respectively, before and during pachytene. The chiasma frequencies fall to 9.2 and 0.9 per cell.

The reduction in chiasma frequency brought about by heat shocks does not take place to the same extent over the whole of the chromosome complement. Short arms (viz. those with a mitotic length of less than

Table 1. *Thirty-five nuclei, untreated, 1937*

	No. of chiasmata											Mean no. of chiasmata	Variance	Variance/ mean	Termi- nization coefficient
	0	1	2	3	4	5	6	7	8	9	10	11			
Bivalent A	.	.	.	.	5	11	9	6	4	.	.	203	5.80	0.262	0.089
Bivalents B and C: Short	.	.	12	21	2	.	.	.	.	.	.	95	2.71	0.122	
Long	.	.	.	1	10	8	9	5	2	.	.	188	5.37	0.307	
Total	.	.	.	.	.	1	5	5	12	6	3	283	8.09	0.287	0.214
Bivalent D: Short	2	26	7	.	.	.	.	.	.	.	.	40	1.44	0.210	
Long	1	4	28	2	.	.	.	.	.	.	.	66	1.89	0.148	
Total	.	2	4	20	9	.	.	.	.	.	.	106	3.03	0.181	0.349
Bivalents E, F, G	.	.	.	.	3	3	8	12	7	2	.	233	6.66	0.177	0.554

Table 2. *Fifty nuclei, untreated, 1938*

	No. of chiasmata															Mean no. of chiasmata	Variance	Variance/ mean	Termi- nization coefficient
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14				
Bivalent A	.	4	8	15	11	11	1	.	.	.	.	170	3.40	1.63	0.479	0.080			
Bivalents B and C: Short	.	1	40	9	.	.	.	.	.	.	.	108	2.16	0.18	0.083				
Long	.	2	6	15	16	8	3	.	.	.	.	181	3.62	1.42	0.392				
Total	.	.	.	1	7	12	18	7	4	1	.	289	5.78	1.60	0.277	0.337			
Bivalent D: Short	1	48	1	.	.	.	.	.	.	.	.	50	1.00	0.04	0.040				
Long	.	18	32	.	.	.	.	.	.	.	.	82	1.64	0.23	0.140				
Total	.	.	19	30	1	.	.	.	.	.	.	132	2.64	0.28	0.106	0.550			
Bivalents E, F, G	.	.	.	.	1	8	15	15	11	.	.	327	6.54	1.15	0.176	0.420			



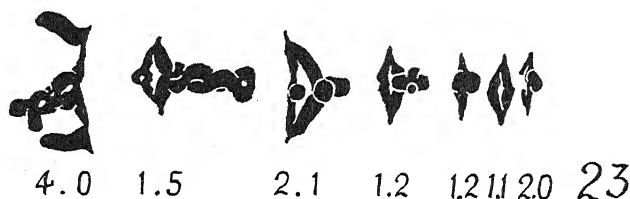
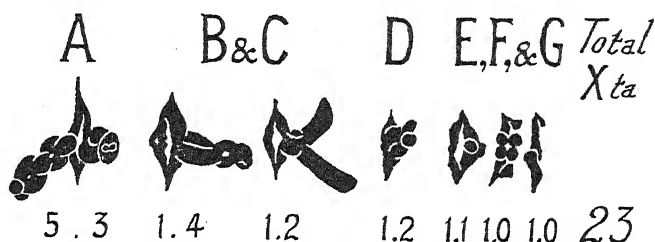
Table 3. *Twelve nuclei, 2 days at 30° C., 9-7 days before meiosis*

	No. of chiasmata										Mean chiasmata	Variance	Variance/ mean	Termina- lization coefficient
	0	1	2	3	4	5	6	7	8	9				
Bivalent <i>A</i>	4	3	5	.	.	.	.	.	.	.	13	1.08	0.81	0.077
Bivalents <i>B</i> and <i>C</i> : Short	.	3	9	.	.	.	.	.	.	.	21	1.75	0.21	
Long	3	6	1	2	.	.	.	.	.	.	14	1.17	1.06	
Total	.	.	6	3	1	2	.	.	.	.	35	2.92	1.36	0.400
Bivalent <i>D</i> : Short	3	9	.	.	.	.	.	.	.	.	9	0.75	0.21	
Long	5	7	.	.	.	.	.	.	.	.	7	0.58	0.27	
Total	.	8	4	.	.	.	.	.	.	.	16	1.33	0.24	0.470
Bivalents <i>E, F, G</i>	.	.	1	3	7	.	1	.	.	.	46	3.83	1.42	0.475

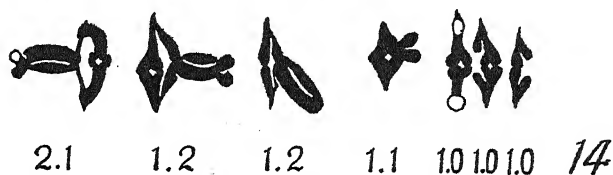
Table 4. *Fifty nuclei, 4 days at 30° C., 8-4 days before meiosis*

	No. of chiasmata				Total chias- mata	Mean chias- mata	Variance	Variance/ mean
	0	1	2	3				
Bivalent <i>A</i>	48	2	.	.	2	0.04	1.000	
Bivalents <i>B, C</i> : Short	33	13	4	.	21	0.42	0.979	
Long	47	3	.	3	3	0.06	1.000	
Total	30	16	4	.	24	0.48	0.875	
Bivalent <i>D</i> : Short	47	3	.	.	3	0.06	1.000	
Long	49	1	.	.	1	0.02	1.000	
Total	46	4	.	.	4	0.08	1.000	
Bivalents <i>E, F, G</i>	36	12	2	.	16	0.32	0.937	

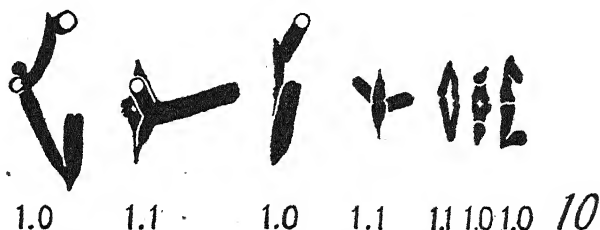
$4\mu$ , mean length  $2.5\mu$ ) suffer far less than the longer (over  $5\mu$  in mitotic length; the mean length of the 5 long arms is  $8.5\mu$ ). This effect is shown



Text-fig. 9.



Text-fig. 10.



Text-fig. 11.

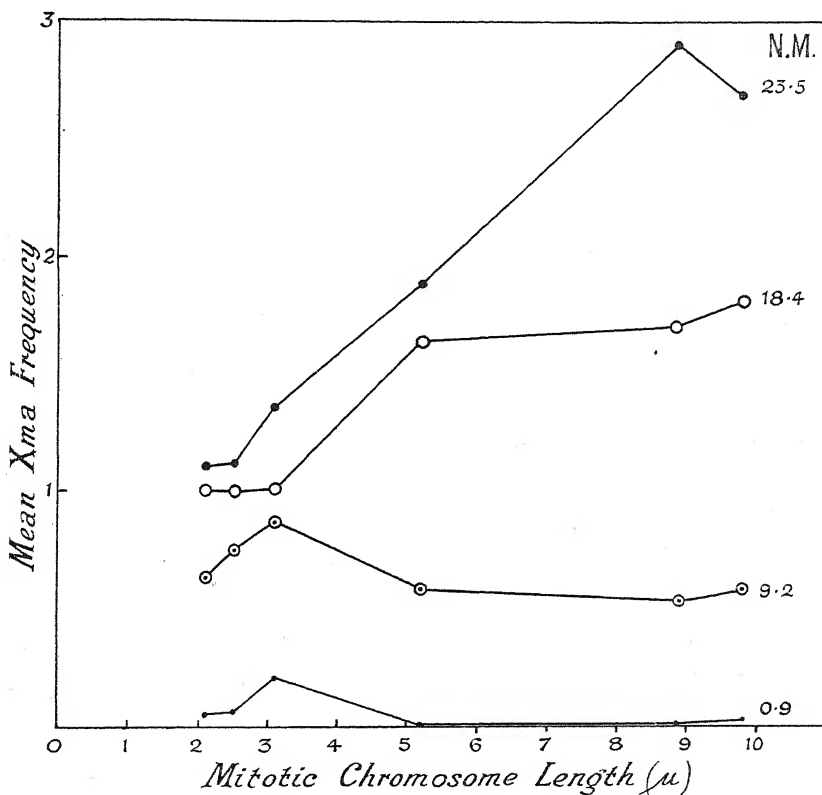
Text-figs. 9-11. The bivalents from four pollen mother cells. Text-fig. 9 shows the bivalents from two cells of the 1937 fixation (nuclear mean 23.5). Text-fig. 10 is from the 1938 fixation (nuclear mean 18.4). Text-fig. 11 is from a plant heated 2 days at  $30^{\circ}\text{C}$ . (nuclear mean 9.2). Note the increasing localization of chiasmata (1) in the short chromosomes or chromosome arms, and (2) at the proximal and distal ends of long arms, with reduction of chiasma frequency. The number of chiasmata in each arm of the bivalents is given below.

in Text-fig. 13, which gives the chiasma frequencies for the separate arms plotted against mitotic length for the four treatments. Where arms



in short arm/long arm ratio are similar to those taking place over the whole nuclear complement, but more extreme.

The increase in localization with reduction in nuclear mean brings about the peculiar result that the longest chromosome, *A*, with no short arms, has the lowest chiasma frequency, when the nuclear mean falls



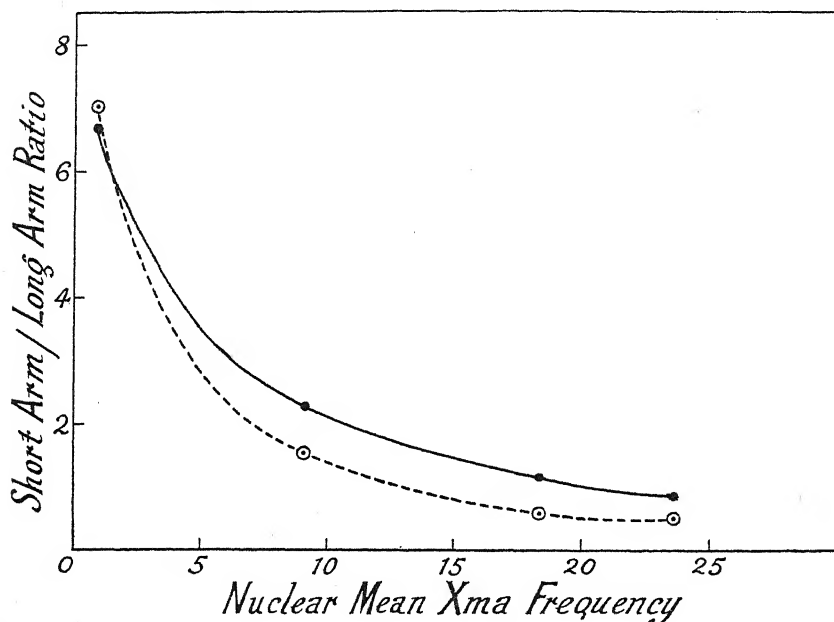
Text-fig. 13. Mitotic length-chiasma frequency curves for the four sets of observations. The nuclear mean chiasma frequency is given opposite each curve.

below ten. Darlington (1936) and Frankel (1940) obtained similar results by comparing the natural frequencies in different *Fritillaria* species. With extreme localization the *M* chromosomes, though longer, have a lower chiasma frequency than the *S* chromosomes.

Thus localization of chiasmata near the centromere can be artificially produced as well as naturally conditioned, and whichever way it happens it affects median chromosomes more than subterminals.

## 5. INTERFERENCE

The frequency distribution of chiasmata shows that the formation of a chiasma within a chromosome interferes with the formation of another in the same chromosome in all species which have been investigated (Haldane, 1931). The apparent magnitude of this type of interference will depend on the regularity of pachytene pairing. An interrupted and



Text-fig. 14. Graph showing increase in number of chiasmata in the short arms per chiasma in the long arms with reduction in nuclear mean. The solid curve is for the whole nucleus; the dotted curve for the subterminal chromosomes (*B* and *C*).

therefore more variable pachytene pairing should bring about a decrease of interference within the chromosome or chromosome arm. We must also consider a further type of interference, namely, competition between different chromosomes in chiasma formation (Mather, 1936). Lastly, there is the question whether the two arms of a chromosome are independent of each other in chiasma formation (Bennett, 1938).

(a) *Interference within the chromosome*

Interference within the chromosome can be measured by the relative variance ( $V/M$ ). The data are given in Tables 1-4. In the untreated plants the relative variance is low but in general increases with decrease

in nuclear mean, until with a nuclear mean of 0.9 chiasmata the mean is equal to the variance in all chromosomes. Thus chiasma formation within the same chromosome or chromosome arm becomes relatively more variable with a lower chiasma frequency. We cannot conclude from this that the true chiasma interference, i.e. the interference presumably depending on the removal of the torsional stress over a short distance of the paired chromosome when a chiasma is formed, is necessarily reduced. Another variable which is, as we shall see, the amount of pachytene pairing enters as the mean chiasma frequency is reduced.

There is another important point. We have seen in § 4 that the long arms suffer to a greater extent than the short arms, when the nuclear chiasma frequency is reduced. The interference values show a similar trend. Thus interference in the short arms does not fall with a small reduction in nuclear mean, e.g. from 23 to 18 chiasmata/cell, whereas in the long arms interference falls with any reduction in nuclear mean. The short arms appear to be more buffered against the effect of environmental conditions.

(b) *Competition between chromosomes*

Table 5 gives the results of an analysis of variance carried out to determine whether there is competition between bivalents in *Uvularia*.

Table 5. *Analysis of variance*

	Degrees of freedom	Sum of squares	Mean square	<i>z</i>	<i>P</i>
Nuclear mean 23.5, 35 nuclei					
Interbivalent	3	487.27			
Internuclear	34	53.44	1.5718	0.0202	<0.05
Inherent	102	153.73	1.5072		
Totals	139	685.44			
Nuclear mean 18.4, 50 nuclei					
Interbivalent	3	521.86			
Internuclear	49	53.38	1.0894	0.0456	<0.05
Inherent	147	175.14	1.1914		
Totals	199	750.38			

The details of the method are given in Mather's (1936) paper. There is no significant difference between the inherent and internuclear variances, and so the chromosomes are independent of one another in chiasma formation.

(c) *The independence of two arms of a chromosome in chiasma formation*

The independence of two distinguishable arms of a chromosome can be tested by means of their correlation coefficient. Table 6 gives correlations for the short arms as opposed to the long in bivalents *B* and *C*.

Table 6. *Correlation between short arms and long arms in chromosomes B and C (from Tables 1-3)*

Thirty-five nuclei; nuclear mean 23.5. Chiasmata in $B_L$ and $C_L$								
	3	4	5	6	7	8	9	Totals
Chiasmata in $B_S$ and $C_S$ {								
2	1	5	1	4	1	.	.	12
3	.	4	7	5	3	2	.	21
4	.	1	.	.	1	.	.	2
Totals	1	10	8	9	5	2	.	35

$$r = \frac{S(xy)}{\sqrt{[S(x^2)S(y^2)]}} = +0.01372; 5\% \text{ point} = 0.324.$$

Fifty nuclei; nuclear mean 18.3							
	1	2	3	4	5	6	Totals
Chiasmata in $B_S$ and $C_S$ {							
1	.	.	1	.	.	.	1
2	1	5	11	15	6	2	40
3	1	1	3	1	2	1	9
Totals	2	6	15	16	8	3	50

$$r = -0.0145; 5\% \text{ point} = 0.273.$$

Twelve nuclei; nuclear mean 9.2					
	0	1	2	3	Totals
Chiasmata in $B_S$ and $C_S$ {					
1	.	3	.	.	3
2	3	3	1	2	9
Totals	3	6	1	2	12

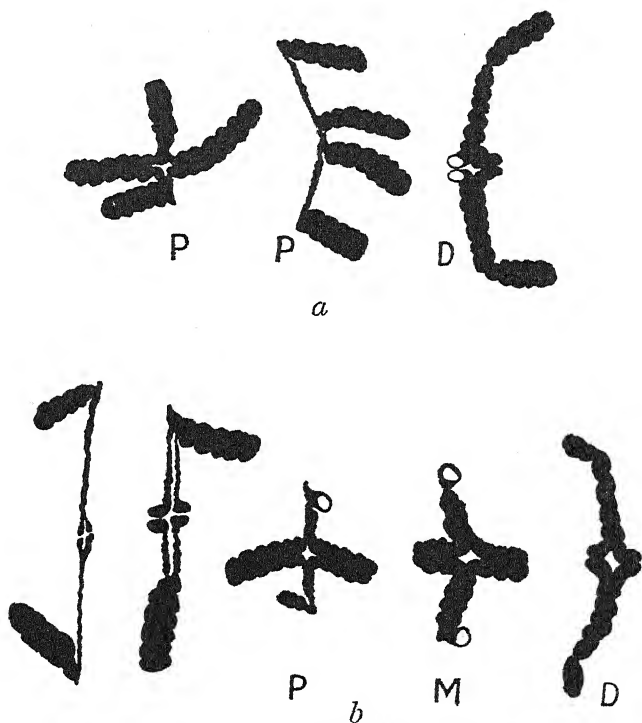
$$r = +0.0118; 5\% \text{ point} = 0.576.$$

None of the values is significant. We can assume that, as *Fritillaria* (Bennett, 1938), the two arms of a chromosome in *Uvularia* are independent in chiasma formation.

## 6. CHIASMA LOCALIZATION AND CHROMOSOME PAIRING

We have seen in § 4 that in *U. perfoliata* when the chiasma frequency is high there is an almost linear relation between chiasma frequency and mitotic length, and an almost even distribution over the length. A linear relation means first that the pairing is complete at pachytene (or that the same proportion of the length of each chromosome is unpaired at the end of pachytene), and secondly, if crossing-over is caused by some torsional stress developed in the paired chromosomes, that the amount of the stress per unit length of paired chromosome is constant. On these

grounds a reduction in chiasma frequency could be brought about in three different ways: (1) by reduction of pachytene pairing, i.e. the agent producing the reduction acting during leptotene or zygotene and before pachytene, (2) by the removal of torsional stress in unpaired parts before pairing is complete, (3) by reduction of the torsional stress throughout the length of the chromosomes paired, i.e. the effective agent



Text-fig. 15. Types of localization (no pairing, *P*, *M* or *D*) in the long arms. The bivalents are from a plant heated at 30° C. for 4 days (acetocarmine). (*a*) median chromosome (*A*); (*b*) subterminal chromosomes (*B* or *C*).

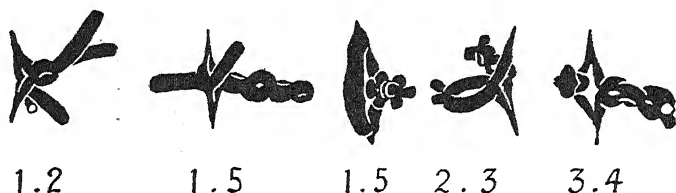
either prevents the development of torsion after pairing or neutralizes it in some way.

If this third hypothesis is the correct one in the present case, it is difficult to see how a localization could be brought about. There is no reason to suppose that long chromosomes should be more affected than short. Any reduction in torsional stress after complete pairing would presumably take place equally over the whole complement.

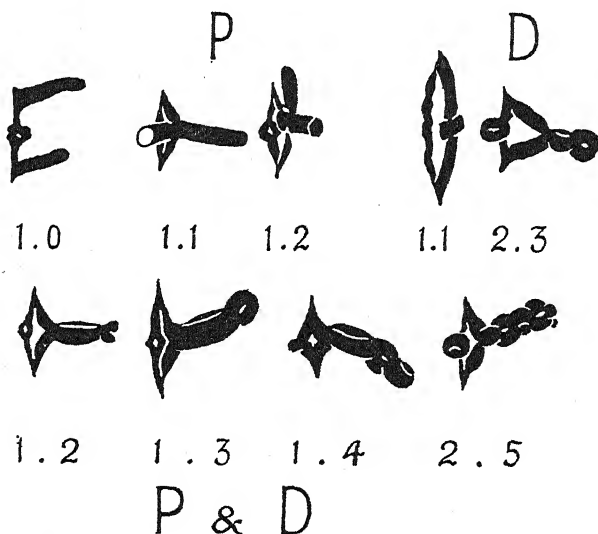
The existence of an effect differential as between long and short arms is, however, easily explained on the first or second hypothesis. A low



nuclear mean chiasma frequency means a smaller amount of pachytene pairing. In effect, the chromosomes have not had enough time in which to pair or if they were slower in starting. We might expect, therefore, that the chiasmata would be localized in those parts which normally pair earliest. Thus in *Uvularia* the shorter the length of a chromosome or



Text-fig. 16. Types of localization in the long arms of bivalent *A*. The bivalents are from plants with natural time limit (nuclear mean 23.5). There is no correlation between pairing types in the two arms.



Text-fig. 17. Types of localization in the long arms of bivalents *B* and *C*. Note pairing always takes place in the short arm. With incomplete pairing the chiasmata in the long arm are usually situated either proximally (*P*) or distally (*D*), or at both places (*P* and *D*). The bivalents in the lower row show stages to complete pairing by closure of the large median loop.

chromosome arm the easier the pairing. We cannot distinguish at present between hypotheses 1 and 2. Probably both are concerned in the greater reduction of chiasma frequency in the long arms in *Uvularia*.

This localization in the short arms follows quite naturally from what we know of the mechanical and spatial relationships of the chromosomes in the resting nucleus. The chromosomes appear at prophase of an

ordinary mitosis in practically the same relative positions as at the previous telophase (see Part I). The centromeres are at one pole with the long arms curling round the nucleus, the ends usually coming nearer together at the opposite pole. The resting nucleus preserves unchanged the spatial distribution of the chromosomes from the preceding telophase (cf. Barber (1940), where it is shown that even the detailed distribution of chiasmata may be retained through a resting stage artificially induced). Thus after the last premeiotic mitosis proximal regions of the chromosomes will on the average be closer together than distal or middle regions. They will, therefore, be in a position to pair earlier. Where the centromere is near an end as in the subterminals and short chromosomes, there will be an added advantage in pairing as the proximal regions will be less anchored than when the centromere is in the middle of a long chromosome. Hence, in particular, chromosomes *B* and *C* have a higher chiasma frequency than chromosome *A* when the nuclear mean is reduced.

#### 7. ZYGOTENE PAIRING IN THE LONG ARMS

We have seen that the short chromosomes or arms start pairing earlier than the long ones. The short arms rarely form more than one chiasma. Long arms when completely paired can form up to six chiasmata. These can be distributed along the arm in various ways, and from the changes in distribution we can follow the sequence of pairing in the long arms.

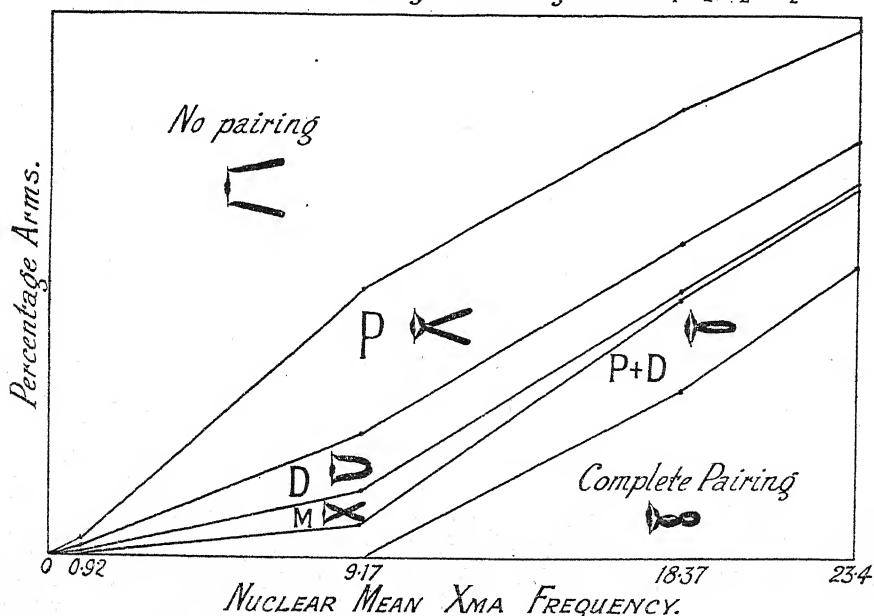
Table 7 shows the chiasma distribution within the long arms (i.e. both arms of *A* and the long arms of *B* and *C*). The same data are plotted graphically in Text-fig. 18. The various classes are distinguishable by whether pairing occurs in the arbitrary proximal (*P*), middle (*M*) or distal (*D*) regions of the arms.

Let us first consider the contact points where initiation of pairing takes place. We must assume, as in the previous discussion on localization between long and short arms, that a low nuclear mean indicates an early interruption in pairing. The points where pairing is initiated are then shown by chromosomes in which little pairing has taken place.

With a nuclear mean of 0.9 chiasmata pairing occurs in the long arms only in about 2% of the cells. Never has more than one chiasma been observed in an arm. The chiasma can occur anywhere along the arm. The actual figures for the distribution of 26 long-arm chiasmata found in about 250 cells are 12 *P*, 5 *M*, 9 *D*. With a nuclear mean of 9.2, chiasma pairing occurs in the long arms in about 50%. The number of chiasmata in an arm never exceeds two. In twelve cells 27 chiasmata

Table 7. *Localization of pairing in the long arms of chromosomes A, B and C. The arms of the A bivalent are scored separately*

	O	P	D	M	PD	PM	DM	C		
$A_1$ and $A_2$									Nuclear mean	Total arms
	2.4	14.4	2.4	2.4	16.8	4.8	4.8	52.2	23.5	42
	21.2	21.2	6.6	—	19.6	1.6	1.6	27.4	18.4	62
	50.0	29.2	8.4	8.4	4.2	—	—	—	9.2	24
	97.4	1.0	1.2	0.4	—	—	—	—	0.9	c. 500
$B_L$ and $C_L$										
	2.4	23.8	7.2	—	14.2	—	2.4	50.0	23.5	42
	14.5	25.8	8.0	3.2	14.5	4.8	1.6	29.0	18.6	62
	50.0	25.0	12.6	4.2	8.4	—	—	—	9.2	28
	97.4	1.4	0.6	0.6	—	—	—	—	0.9	c. 500

*Localisation of Pairing in the Long Arms.  $A_1, A_2, B_L$  &  $C_L$ .*

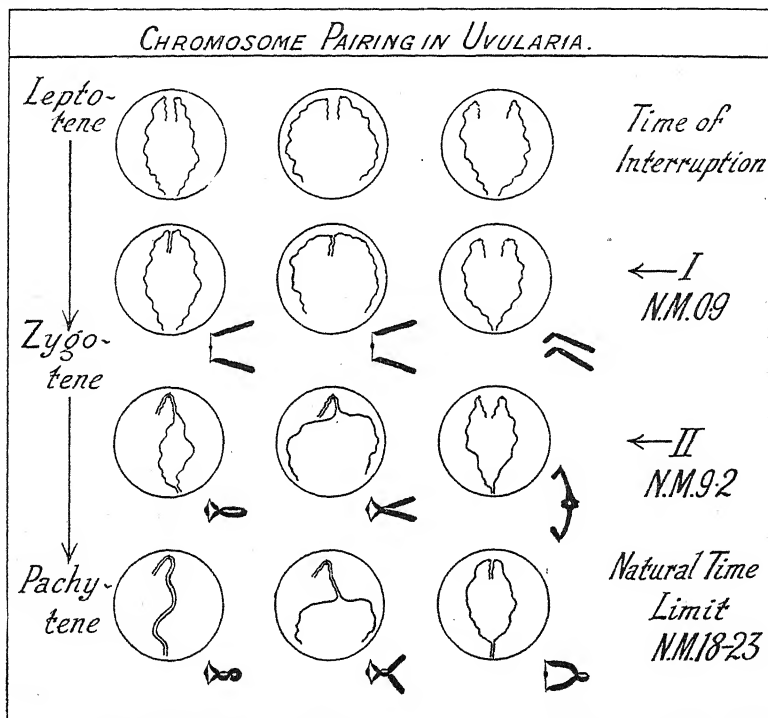
Text-fig. 18. Graphs illustrating changes in localization of pairing in the long arms with change in nuclear mean chiasma frequency. The data are taken from Table 7 with both subterminal and median arms grouped.

were formed in the long arms. These were 16 *P*, 3 *M* and 8 *D*. These two sets of figures agree in showing a moderate localization of chiasmata in the end regions of the long arms. The localization is greater in the region near the centromere. Pairing in the long arms is therefore as a rule initiated proximally or distally. It can start at both ends in the same arm, as is shown by the arms with two chiasmata which have one proximal and one distal chiasma (the *P* and *D* class).

With a further increase in chiasma frequency, as for instance with pairing under natural time limits, a large increase is found in the number of chiasmata in all three regions, but especially in the *M* region. Table 7, which gives the pairing regions, shows that this increase in the *M* chiasmata is brought about largely by arms becoming fully paired, that is by forming at least one chiasma in each of the three regions. Arms with a single chiasma or two chiasmata close together in the *M* region are rare. Thus, pairing does not usually take place in the *M* region unless both ends are first paired. This is also shown by the small size of *PM* and *DM* classes. The *PD* class on the other hand is relatively large. Thus the sequence of pairing usually seems to be: initiation proximally and distally followed by a spread of pairing into the middle regions. All stages in reduction of size of the middle loop can be recognized (see Text-fig. 17). If, on the other hand, there is only a single distal or proximal contact point, the arm remains incompletely paired even under the natural time limit. Text-figs. 16 and 17 show bivalents in which pairing probably started proximally and distally respectively. The pairing in each case has not had time to reach the end opposite to that in which pairing began.

Complete pairing in a long arm therefore depends on pairing being initiated in at least two points of its length. Under the natural time limit, pairing does not travel along the arm rapidly enough for a single contact point to lead to complete pairing. This marginal rate of travel has other important consequences. In Table 7 the pairing regions for both the median and subterminal types of long arm are given separately. Localization is almost the same in both types. Although pairing in the short arm of the subterminal chromosome begins before that in the long arm, it only slightly increases proximal pairing in the long. Similarly the types of localization in the two arms of the *A* chromosome are not correlated. For instance, one arm may be completely paired whilst the other may show no pairing (e.g. Text-fig. 9*A*), or a proximal localization, or a distal (Text-fig. 16). Thus a proximal pairing does not seem to pass over the centromere into the other arm to any great extent. The two

arms of chromosomes are virtually independent in pairing. This is the reason for the absence of a correlation between chiasma frequency in the two arms of the subterminal chromosomes which was demonstrated in the last section. Further, in contrast to *Fritillaria* (Darlington, 1936; Frankel, 1940), the subterminal long arms do not show a considerably higher chiasma frequency than the median long arms of chromosome *A*,



Text-fig. 19. Diagram of the pairing of a subterminal chromosome (*B* or *C*) in *U. perfoliata*. Three extreme types are shown, depending on the spatial distribution of the chromosomes in the leptotene nuclei. The first is the most frequent in *Uvularia*, occurring in about 70 % of the nuclei; the second occurs in about 20 %. Distal pairing alone occurs in less than 10 % of the nuclei. The types of metaphase bivalents arising from these pairing types are shown below each, and the times at which pairing is interrupted, naturally or artificially, are indicated.

when pairing is interrupted at an early stage (see Tables 1-4). The earlier pairing of the short arm does not lead to earlier pairing of the attached long arm in *Uvularia*.

Let us consider the probable causes for the localization of pairing in the long arms. We have seen that short arms pair before the long because of their greater freedom of movement and closer proximity in the leptotene nucleus. Similar factors probably control the localization in the

long arms. We might expect proximal and distal regions to be closer together than the remainder because the chromosomes will enter the premeiotic resting stage with the centromeres at one pole of the nucleus and the ends of the long arms at the opposite pole. These regions will thus have an advantage in pairing. The arrangement of the centromeres will on the whole be more regular than that of the ends. Possibly this is responsible for the greater degree of proximal localization.

I have attempted in the diagram (Text-fig. 19) to show schematically the course of pairing in a subterminal chromosome in *Uvularia*. Three extreme types of chromosome arrangement in the leptotene nucleus are shown, together with the types of bivalent arising under the artificial and natural time limits to pairing.

### III. CO-OPERATION AND BALANCE IN POLLEN GRAINS

#### 1. INTRODUCTION

The possession of a full complement of chromosomes is in general necessary for the division and functioning of a pollen grain. Subhaploid grains almost always degenerate and die before the first post-meiotic mitosis. A few cases of division have been found by Levan (1936, 1937) in *Allium*. Belling also found a single instance of division in an ( $n-1$ ) grain in *Uvularia grandiflora*. It was, exceptionally, attached to its complementary ( $n+1$ ) grain, the two grains being at the same stage of development. Belling thought that his failure to find more divisions in deficient grains was due to their development being slower. We shall see that this is not the case. The present paper gives an account of experiments which show that division in the deficient grains can take place only when these grains remain attached to their complementary hyperploid grains.

#### 2. EFFECTS OF HIGH TEMPERATURES ON THE MEIOTIC DIVISIONS

The effects of high temperatures on the meiotic divisions are of two kinds:

(a) Interruption of pachytene pairing, with consequent failure of metaphase pairing (see Part II) and

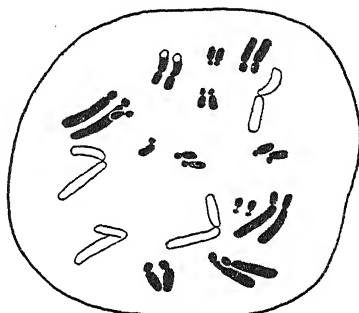
(b) Suppression or incomplete development of the spindle. In either case polyploid and unbalanced pollen grains are formed.

Interruption of pairing leads eventually to the formation of univalents. Thus for example 4 days at 30° C. during the prophase of meiosis reduces the chiasma frequency of *U. perfoliata* from about 20 per

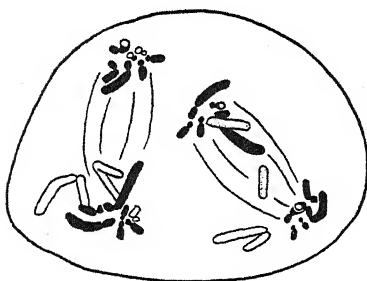
cell to 0.9 per cell (see Pl. 16, fig. 1). Shorter treatments have less effect. The univalent chromosomes usually become orientated (Pl. 16, fig. 2) but apparently do not often divide at the first division, since de-spiralization starts before this can occur (Pl. 16, fig. 3). With a large number of univalents as in these cells, cell division is usually suppressed and the nuclei may fuse to form a single large restitution nucleus. In other cases the nuclei remain separate but within a single cell; or where there are



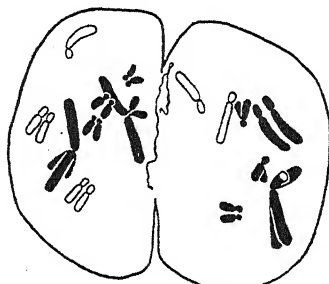
Text-fig. 20.



Text-fig. 21.



Text-fig. 22.



Text-fig. 23.

Text-figs. 20-23. Second metaphases in pollen mother cells from plant heated 4 days at 30° C. Further explanation in the text.  $\times 850$ .

only one or two pairs of univalents, cell division takes place normally, with the univalents sometimes remaining as micronuclei.

Text-figs. 20-23 give examples of pollen mother cells at the second division after an interrupted pairing (chiasma frequency at first metaphase on the same slide is 0.9 per cell). Text-fig. 20 is a practically normal second metaphase, except that although there are apparently two spindles, the pollen mother cell is undivided, and also one of the *A* chromosomes is off the plate and unorientated. Presumably this will give rise to  $(n-A)$  and  $(n+A)$  pollen grains.

Text-fig. 21 shows a cell in which a restitution nucleus has been formed. The *A*'s must have divided as univalents at the first division, since the four chromatids are widely separated. Spindle formation is abnormal and the centromeres, although divided, do not seem to be drawing the chromatids apart: lapse into the resting stage will give a tetraploid cell with most of the chromosomes paired at the pollen grain division (cf. Text-fig. 26).

Text-fig. 22 is similar, but the chromosomes are orientated on two spindles within the same cell. The *A* and *E* chromatids show random segregation, presumably owing to division of their univalents at first metaphase. One of the *A* chromatids is fragmented at the centromere and the two halves appear to be moving apart. In two other cells similar fragmentation has been found. It is probably caused by misdivision of the centromere (Darlington, 1939).

Text-fig. 23 illustrates a further abnormality at second metaphase. The *D* chromosomes did not disjoin at the first division. Two of the pollen grains will be deficient for this chromosome. The *C* chromosomes divided as univalents at the first division and one chromatid, caught up in the wall, has remained unspiralized.

These four cells show most of the abnormalities found at second division in the heated plants of *Uvularia*. From them polyploid and unbalanced pollen grains will be formed. It is the purpose of the present paper to show the conditions of survival of some of these types.

### 3. EFFECTS OF HEAT ON POLLEN-GRAIN DEVELOPMENT

Most of the heated plants were taken out of the incubators before or immediately after the meiotic divisions. Some, however, were left at the high temperatures till after the pollen-grain mitosis. In the first case pollen-grain development, except for those effects which are caused by meiotic upsets, e.g. failure of chromosome pairing or abnormal spindle development, is normal. The four grains in the tetrad separate and usually develop the characteristic shape with a fairly thick finely punctate wall. Many of the grains fail to develop up to the time of the pollen grain mitosis and remain as empty shells. As we shall see, these are probably the unbalanced and deficient grains. In many of the experiments over 50% of the grains are empty and dead by the time the first mitosis should take place.

If, on the other hand, the high temperatures are continued up to the time of the first pollen-grain mitosis, the four grains in the tetrad fail to separate. The grains remain more or less spherical, instead of becoming



oval as in normal plants. Further, wall formation is abnormal. The walls remain very thin and delicate and often do not completely separate the four grains of a tetrad from one another. The figures (27-35 and Pl. 16, figs. 3-5) give examples of the unseparated tetrads at the time of the first pollen-grain mitosis. They were obtained from a plant heated at 25° C. for 4 weeks, the treatment beginning a fortnight to 3 weeks before meiosis. Most of them (over 80 %) appear to survive up to the time of the first pollen-grain mitosis. Empty grains are rare.

Continued high temperatures have a similar effect on wall formation in other species, e.g. *Fritillaria Meleagris*. When plants of this were placed in an incubator at 30° C. just as the meiotic divisions were taking place, normal tetrads were formed, but the cells failed to separate. Wettstein (1924) has shown that injection of anaesthetics into moss capsules delays separation of the cells in a tetrad. He was able to use this technique for an investigation of tetrad segregation. It may be possible to use the heat technique in the same way.

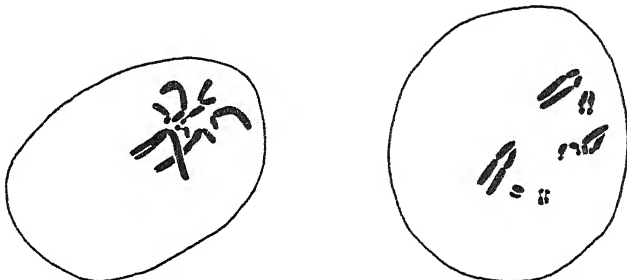
#### 4. THE POLLEN-GRAIN DIVISION

We have seen that an abnormal disjunction of the chromosomes occurs in heated plants of *Uvularia*. Unbalanced, deficient and polyploid pollen grains are produced in large numbers. Table 8 gives the numbers of chromosomes in pollen grains of heated plants at the first post-meiotic mitosis. Where the pollen grains separate, over 80 % of the grains dividing are haploid, diploid or tetraploid, i.e. they are balanced types. A few unbalanced grains were noticed, but always (with one exception), hyperploid. The three grains with 8 chromosomes had an extra *B* or *C* chromosome (see Text-fig. 24). One grain (Text-fig. 25) had only six complete chromosomes and a short fragment. The missing chromosome is the *E*, one of the three shortest. This was the only case observed where a deficient grain separated from its complementary grain survived till the pollen-grain mitosis. Probably it survived because the fragment represents most of the missing *E* chromosome. The cell was abnormal in that the chromosomes were supercontracted and not orientated properly, although the centromeres were apparently divided.

The diploid and tetraploid grains often show pairs of homologous chromosomes lying parallel. Belling (1925) illustrated such somatic pairing in diploid pollen grains obtained by cold treatment. The pairing results from suppression of the anaphase movement at one or both meiotic divisions (see Text-fig. 22). Text-fig. 26 shows an example with

twenty-four chromosomes ( $3x+3$ ). All the chromosomes, except two of the *D*'s, are paired, the centromeres being closest together.

Chromosome counts from tetrad pollen show a different distribution. Deficient grains survive and undergo the first pollen mitosis. Text-fig. 27 shows a tetrad in which two of the cells are at metaphase. One cell, in-



Text-fig. 24.

Text-fig. 25.

Text-fig. 24. Metaphase in  $n+1$  pollen grains. There is an extra *C* chromosome.  $\times 850$ .

Text-fig. 25. Early anaphase in a pollen grain with 6 chromosomes and an acentric fragment. The *E* chromosome is missing.  $\times 850$ .



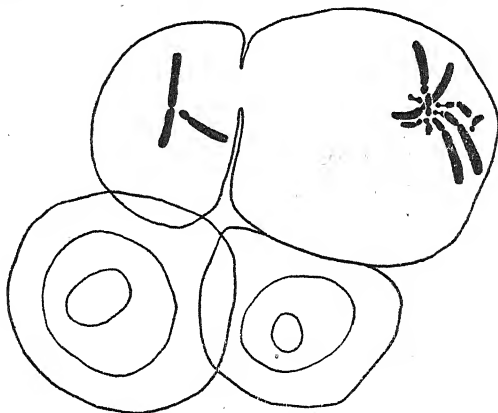
Text-fig. 26. Metaphase in pollen grain with 24 chromosomes (i.e.  $3x+3$ ). Two *A*'s and two *F*'s are missing from the tetraploid complement. Note the paired arrangement of the centromeres, except for one pair of *D*'s.  $\times 850$ .

completely separated from its fellow, has only two chromosomes; the other cell has twelve. Other examples are given in Text-figs. 28-35. In most of them the wall separating the two cells is incomplete and the two nuclei are in protoplasmic connexion. They are similar to the cells illustrated by Belling (1925).

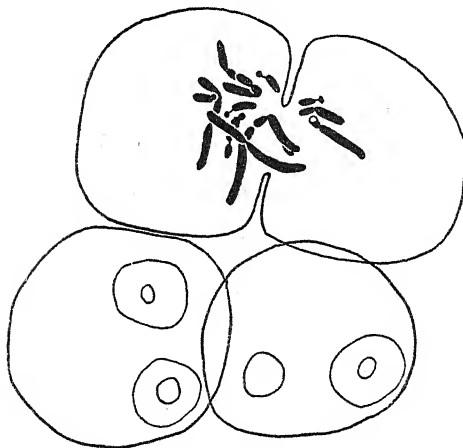
The survival of deficient grains in the tetrad pollen but not in the single pollen grains shows conclusively that the cells in a tetrad must

co-operate in development. A deficient nucleus or grain survives when in contact with a hyperploid grain but not if separated from it.

This co-operation in development shows itself in another way. All the tetrads illustrated show two cells at the same stage of development.



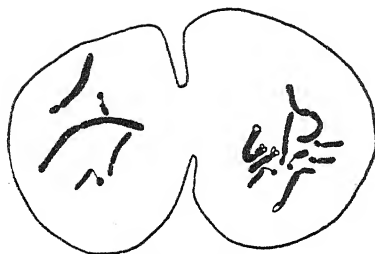
Text-fig. 27.



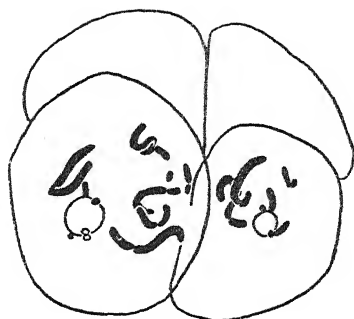
Text-fig. 28.

Text-figs. 27, 28. Two tetrads showing division in deficient cells attached to their complementary hyperploid grain. The chromosome constitutions of the nuclei are given in Table 10 (tetrads 1 and 2). In Text-fig. 28 the sub-haploid nucleus is apparently close to the hyperploid nucleus. They were, however, at different focal levels, the chromosomes being orientated on two separate spindles.  $\times 850$ .

For example, Text-figs. 27, 31, 32, etc., show two metaphases in adjacent cells; Text-fig. 30, two mid-prophases; Text-fig. 35, two telophases, etc. In Text-fig. 34, both cells have the haploid complement of seven chromosomes, but both show the same abnormality in spindle development.



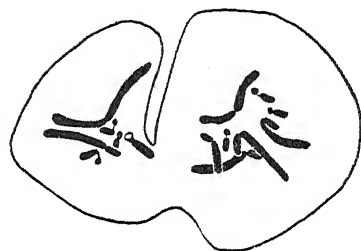
Text-fig. 29.



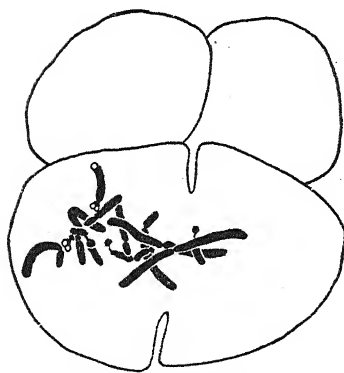
Text-fig. 30.



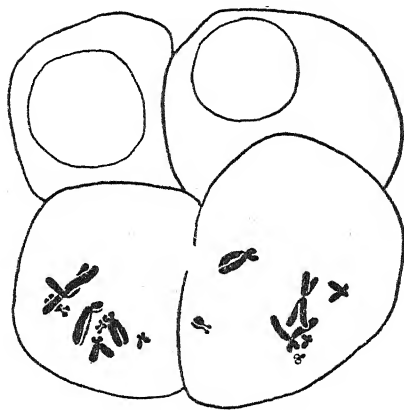
Text-fig. 31.



Text-fig. 32.



Text-fig. 33.

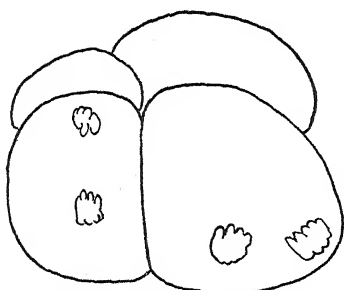


Text-fig. 34.

Text-figs. 29-34. Tetrads and half-tetrads showing synchronization in division of two chromosomally complementary nuclei. The constitutions are given in Table 3, tetrad numbers 3, 4, 6, 7, 8, 9 respectively. Text-fig. 29 shows two late prophases, Text-fig. 30 two mid-prophases with nucleolar chromosomes *F* and *G* still attached to their nucleoli; and Text-fig. 34, two delayed metaphases. The remainder are at metaphase.  $\times 850$ .

The chromosomes are super-contracted and unorientated. Although both cells are balanced, again we see that the unit in development seems to be the pair, not an individual cell or nucleus.

Table 9 gives quantitative data on the synchronization of development in the tetrads. It will be seen that the two cells which are syn-



Text-fig. 35. Tetrads showing two synchronized telophases.  $\times 850$ .

Table 8. *Pollen-grain counts: Uvularia*

Chromosome no.	2	3	5	6	$x$					$2x$			$4x$		%
					7	8	9	11	12	13	14	24	28	Totals	balanced
Tetrad pollen; nuclei counted separately	1	1	3	2	4*	4	1	2	1	1	.	.	1	21	14.3
Tetrad pollen; synchronized nuclei counted together	.	.	.	.	.	.	.	.	2	7	1	1	11		72.7
Independent pollen grains	.	.	.	1†	15	3	.	.	.	.	4	1	3	27	81

\* One pair has  $7 - A + C$  and  $7 + A - C$ .

†  $-E + \text{fragment}$ .

Table 9. *Synchronization of development of the cells of tetrads.*

$D = \text{dividing cell}$ .  $R = \text{resting cell}$ .

21	4	1	12	0

chronized are always along one of the sides of the cruciate tetrad, never diagonally placed, because in the latter case there is insufficient contact. They often appear to be the two sister cells derived from the second division. Occasionally only one cell in the tetrad (or diad) divides. These

cells appear to contain more than the diploid number of chromosomes, which may account for their non-co-operative development. Thus the unit in development usually appears to be a pair of contiguous cells in a tetrad. Table 8 shows immediately why this is so. We have seen that the nuclei arising after heat treatment are usually unbalanced. Co-operation between two cells leads to an increase in balance (Table 8 A). Thus, if we take as our unit the two dividing nuclei in a tetrad, over 70 % of the tetrads showing division are balanced,  $2x$  or  $4x$ , whereas if we take the dividing nuclei separately only 14.3 % are balanced.

Table 10 gives a complete analysis of ten pairs of cells or nuclei which were synchronized in development. It will be seen that in seven cases out of ten the two co-operating nuclei are exactly complementary and together make up the diploid complement. Usually one of the nuclei is

Table 10

Chromosome	1st nucleus							Total	2nd nucleus							Total	
	A	B	C	D	E	F	G		A	B	C	D	E	F	G		
Tetrad no. 1	1	.	1	.	.	.	.	2	1	2	1	2	2	2	2	12	$2x$
2	.	1	.	1	.	.	1	3	2	1	2	1	2	2	1	11	$2x$
3	1	.	1	1	.	1	1	5	1	2	1	1	2	1	1	9	$2x$
4	.	1	1	.	1	1	1	5	2	1	1	1	1	1	1	8+f	$2x-D+f$
5	.	1	1	1	.	1	1	5	2	1	1	1	1	1	1	8	$2x-E$
6	1	1	1	.	1	1	1	6	1	1	1	2	1	1	1	8+f	$2x+f$
7	1	1	.	1	1	1	1	6	1	1	2	1	1	1	1	8	$2x$
8	2	1	.	1	1	1	1	7	.	1	2	1	1	1	1	7	$2x$
9	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	7	$2x$ , both haploid
10	.	4	2	1	.	4	.	11	4	.	1	2	3	.	3	13	$(3x+3)$
Total non-disjunctions (10 excluded)	4	2	3	3	4	2	1										

deficient for one or more chromosomes which are present in duplicate in the other nucleus, although cases do occur (tetrads 8 and 10) in which both nuclei are deficient, but deficient for different chromosomes.

Of the three exceptional pairs, one lacks one of the  $E$  chromosomes, another lacks the  $D$  but possesses a small fragment which possibly represents part of the missing chromosome. The third exception has 24 chromosomes in all (i.e.  $3x+3$ ). One of each of the  $C$ ,  $D$  and  $E$  chromosomes and an  $F$  or  $G$  are missing from the complete tetraploid complement. However, both nuclei are deficient and the co-operation again leads to an increase in balance.

There remains the further problem of whether all types of deficient nuclei are equally capable of co-operating with their complementary hyperploid nuclei. Within the nine approximately diploid pairs, cases of non-disjunction of every one of the seven chromosomes are found. The

*A* and *E* chromosomes suffer non-disjunction four times, the others from one to three times. The pair of cells illustrated in Text-fig. 27 show that the possession of a nucleolar organizing chromosome is not necessary for the survival and division of a deficient nucleus under these conditions. The deficient nucleus consists only of the *A* and *C* chromosomes, neither of which possesses a nucleolar organizer.

#### 5. BALANCE AND REGULATION IN THE POLLEN GRAIN

We have seen that the two resting nuclei in the half-tetrads influence one another so that both survive to the first pollen-grain mitosis. Separated, one or both would die. This is, of course, another proof that the resting nucleus is physiologically active (cf. Stern, 1937; Darlington, 1937*b*). Each nucleus can make good the deficiency of the other, so that together they make a balanced system.

Such co-operation is unusual among plant cells. For instance after X-raying of somatic tissues, the unbalanced and deficient types of cell produced are for the most part eliminated in succeeding divisions. The genetically different types of cell compete with one another, the normal type being best adapted. It is also unusual for micronuclei in pollen grains to develop normally even if accompanied by a normal haploid nucleus. They degenerate, forming "extranuclear bodies", "chromatin-klumpen" (Levan, 1936), etc., at metaphase of the division of the main nucleus. Competition rather than co-operation takes place. Clark has, however, described the behaviour of micronuclei in a mutant maize, which is similar in some respects to that in *Uvularia*. Meiosis is affected so that single nuclei are not formed at telophase, but each pollen grain contains several micronuclei. Most of the pollen grains degenerate, but some undergo the post-meiotic divisions and may be effective in pollination. Division occurs only when the complete haploid complement is present in the several micronuclei in a single pollen grain. Co-operation in development takes place. Similar cells also occur in the tetraploid *Primula kewensis* (Upcott, 1939*b*), arising from split spindles at meiosis. The deficient nucleus, which may contain from 1 to 9 chromosomes ( $2n=36$ ), is always exactly synchronized in development with the major nucleus and together they usually make up the balanced complement.

Thus it appears from comparison of these types of development that two resting nuclei in the same cell or in cells in protoplasmic connexion only co-operate in development when such co-operation leads to an increase in balance. Where co-operation would lead to a decrease in balance of one of the two, it does not take place. The more balanced

nucleus divides, the unbalanced degenerates. An  $(x+1)$  or an  $(x+2)$  nucleus of a pollen grain can usually divide, but in a binucleate pollen-grain with  $x$  chromosomes in one nucleus and the other deficient, only the  $x$  nucleus divides. It is, if we like, an example of regulation depending on the reaction—co-operation or competition—between the two resting nuclei.

#### SUMMARY

##### I. THE CENTROMERE AT MITOSIS

1. The mitotic chromosomes of *Uvularia perfoliata* are described.
2. The centromere is conspicuously bipolar when orientated at the mitotic metaphase. This bipolarity is not developed in non-orientated chromosomes.
3. Recurrent non-orientation of one particular chromosome indicates genetic variability in the efficiency of the centromeres of the different chromosomes.

##### II. THE SEQUENCE OF PAIRING AT MEIOSIS

1. In *Uvularia perfoliata*, temperature shocks during early prophase of meiosis reduce the chiasma frequency.
2. The reduction affects long chromosomes or arms to a greater extent than short ones. The chiasmata become more localized in the short chromosomes or arms with reduction in chiasma frequency.
3. With reduction in chiasma frequency in the long arms chiasmata become localized at both ends, to a greater extent proximally than distally.
4. These two types of localization show that the reduction in chiasma frequency is brought about by an *interruption* of pachytene pairing. Pairing in the chromosomes normally begins (*a*) in the short arms and then (*b*) at both ends of long arms.
5. This localization of pairing depends on two factors, (*a*) proximity of certain regions of the chromosomes, which is determined by persistence of the telophase arrangement from the last premeiotic mitosis, and (*b*) a greater freedom of movement of short chromosomes or the ends of long ones.

##### III. CO-OPERATION AND BALANCE IN POLLEN GRAINS

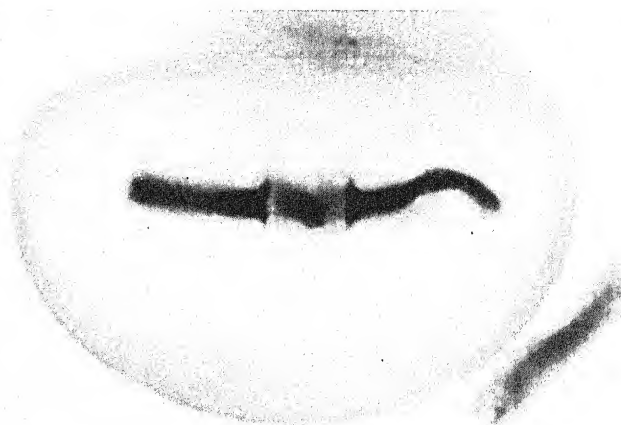
1. In *Uvularia* the application of high temperatures during meiosis leads to the formation of polyploid and unbalanced pollen grains.
2. Deficient pollen grains if separate die, but they survive to the first pollen-grain mitosis if they remain attached to their complementary grain.



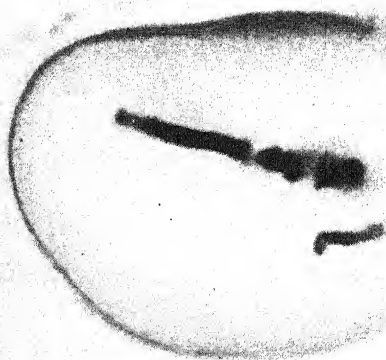
The two nuclei are always exactly synchronized in development and appear to co-operate.

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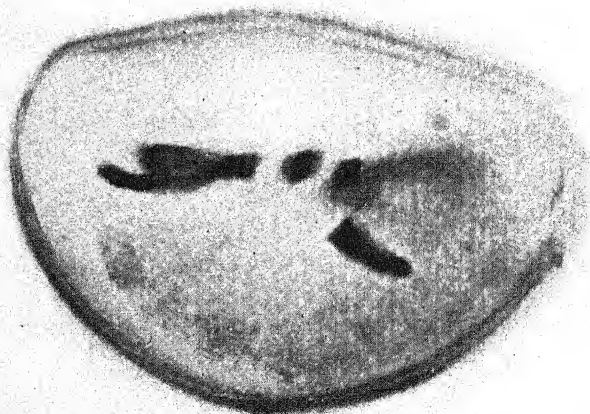
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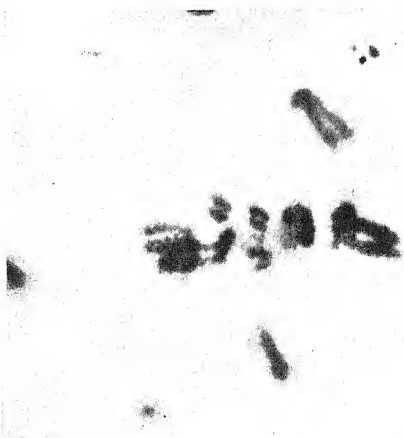


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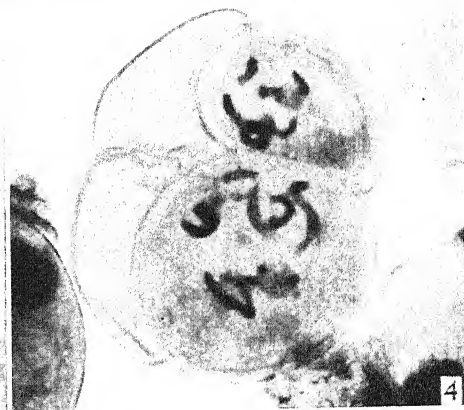
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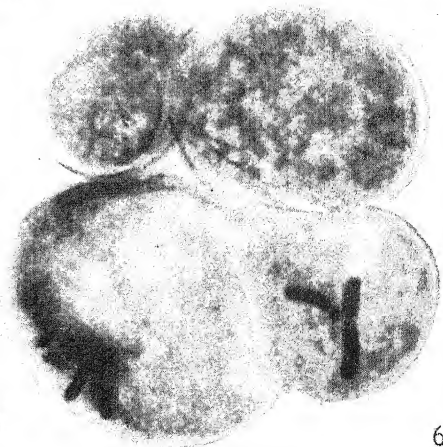
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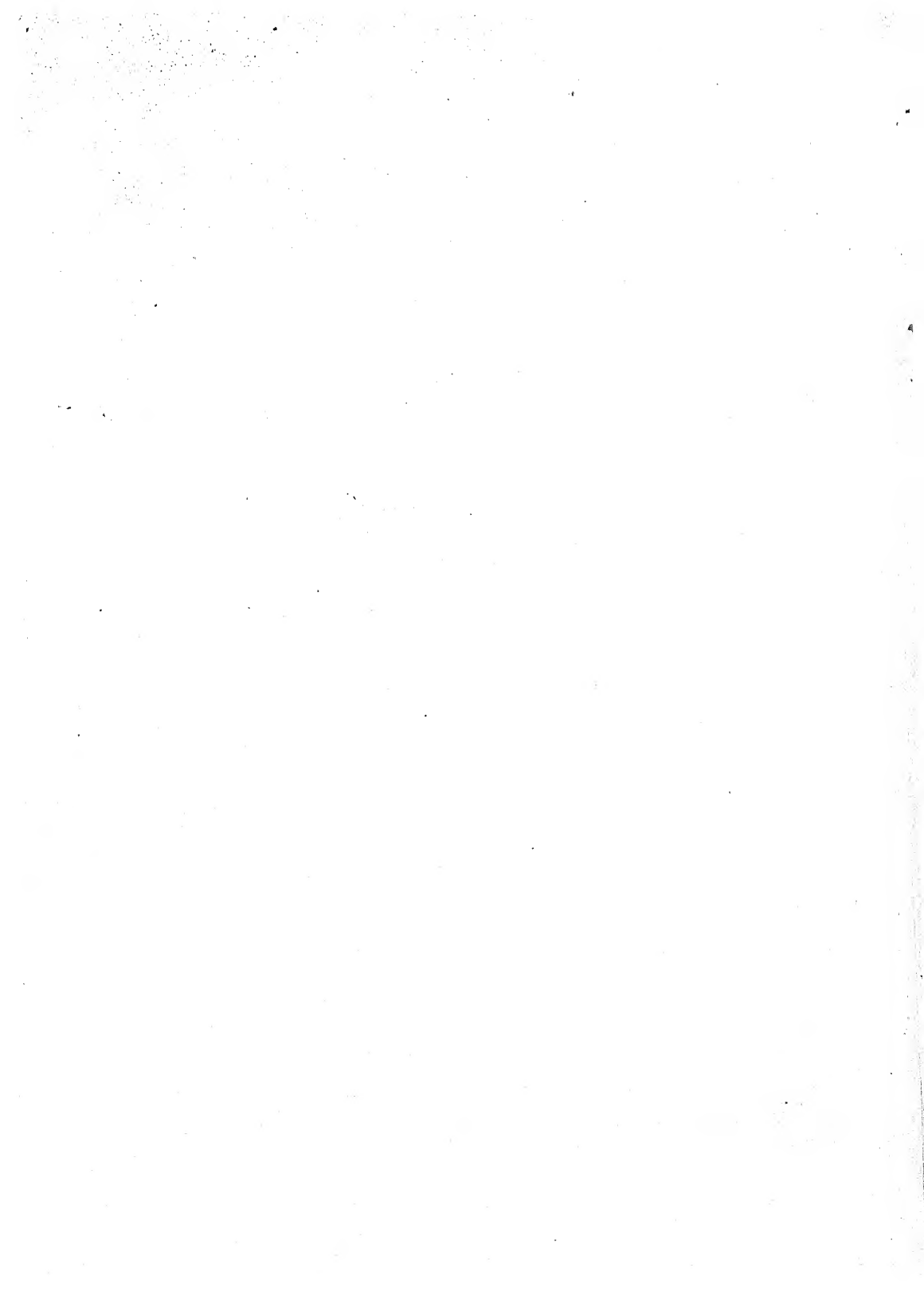
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## EXPLANATION OF PLATES 16 AND 17

## PLATE 16

- Fig. 1. Side view of metaphase in pollen grain, showing co-orientated daughter centromeres. Only two pairs are in focus.  $\times 1500$ .
- Fig. 2. Side view of metaphase showing *D* chromosome off the plate, but orientated on the spindle.  $\times 1500$ .
- Fig. 3. Side view of metaphase with *E* chromosome off the spindle. Its centromeres do not show the repulsion characteristic of the chromosomes on the plate.  $\times 1500$ .

## PLATE 17

- Fig. 1. Cell at early anaphase from plant heated at 30° C. for 4 days. There are four bivalents just separating and four univalents (*A* and *F* chromosomes) in focus.  $\times 1000$ .
- Fig. 2. Anaphase in cell with one bivalent (*B* or *C*). The univalents have all orientated on the spindle.  $\times 1000$ .
- Fig. 3. Telophase from heated plant. The univalents orientated but despiralized before their division.  $\times 1000$ .
- Fig. 4. Tetrad from plant heated 4 weeks at 25° C. Two of the cells are at mid-prophase, the other two empty. Note the nucleolar chromosomes still attached to their nucleoli. The cell is drawn in Text-fig. 30.  $\times 750$ .
- Fig. 5. Delayed metaphases in heated plant. The cell is drawn in Text-fig. 34.  $\times 750$ .
- Fig. 6. Tetrad showing division in deficient cell ( $n=2$ ) with division in its complementary hyperploid cell.  $\times 750$ .



# THE COMPARATIVE GENETICS OF *GOSSYPIUM ANOMALUM* AND THE CULTIVATED ASIATIC COTTONS

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(With Plate 18)

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## I. INTRODUCTION

*GOSSYPIUM ANOMALUM* Wawra & Peyr. is a distinct and truly wild species with a very wide distribution in Africa. As far as is known it is confined



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to arid steppe country, in two apparently discontinuous areas. The first of these is on the southern borders of the Great Desert Belt, where the species has been recorded from as far west as the Hombori district south of Timbuktu, from the Damergou region south of Air, from Kordofan in the Anglo-Egyptian Sudan, to as far east as Somaliland and Abyssinia, north to Nubia. In parts of this area it is said to provide valuable fodder for camels. The other area of occurrence is the dry coastal belt in Angola and South-West Africa, from Loanda in the north, southwards as far as Damara Land. Thus its general distribution considerably overlaps that of *G. arboreum* and *herbaceum*, the two cultivated Asiatic cotton species, but localities are not known with any great exactitude. From the marked xerophytic habit of *anomalum* as contrasted with the mesophytic character of the cultivated cottons it appears unlikely that they occupy the same ecological areas, though in country of the steppe type their habitats might not necessarily be far removed from one another.

This species has been variously described under the names *G. microcarpum* Welw., *G. senarense* Fenzl., and *G. herbaceum* var. *Steudneri* Schweinf., and has even been transferred to the genus *Cienfuegosia* by Schumann as *pentaphylla* and by Gürke as *anomala*, on the basis of its small linear bracteoles, well-marked calyx teeth, and tricarpellary ovary. The morphological grounds for its retention in *Gossypium* have been adequately discussed by Chevalier (1933), who collected material near Damergou and sent seed to this and several other experiment stations in 1932. Later, Chevalier (1935) felt justified in his conclusions on receiving information that the species had been crossed with both Old and New World cottons by a Russian worker, and that second generation hybrids were in existence. No further reports on the behaviour of these hybrids have followed this announcement.

*G. anomalum* is not of direct commercial importance since the hairs on its seeds are only 8–10 mm. long, sparse and dark brown in colour, and non-expansive, remaining compactly adherent to the testa. Its particular interest lies in the fact that it is the only wild species which has given any fertility in hybrids with the cultivated Asiatic cottons, so that it has been possible to analyse its genetic constitution in terms of that of the latter. Like *arboreum* and *herbaceum*, *anomalum* is also a diploid species, with  $n=13$  (Skovsted, 1934). The results of this analysis, together with the evolutionary implications, form the subject of the present paper. They give confirmatory evidence of the general validity of Harland's (1936) conclusions on the formation of species in *Gossypium* by divergence of alleles, duplicates, and modifier backgrounds. Harland's

thesis has been established almost entirely upon crosses within the tetraploid New World complex of species, which form a relatively homogeneous group, so much so that their hybrids are fully fertile, though that they are also good species is shown by the breakdown of viability and fertility in  $F_2$ . The two cultivated Asiatic species *arboreum* and *herbaceum* are similarly quite closely related, giving a fully fertile  $F_1$ , and breakdown in  $F_2$ , but in most characters they show much less modifier segregation than New World interspecific hybrids. On the other hand, *anomalum* has been found to be so distinct from the cultivated Asiatics that its hybrids are almost sterile, and early generations from them give highly complex segregation which completely defies analysis. By repeated backcrossing into the two cultivated species it has been possible to sort out particular factors into different lines on stable backgrounds, and later by synthesis some of the complex types of earlier generations were re-established and their constitution demonstrated. Relatively little backcrossing to *anomalum* was performed, not only because in any case its whole genotype was unknown, but also because of its slow growth and uncertain cultivation under the highly humid conditions in Trinidad. Moreover, seed when obtained was difficult to germinate on account of its very hard testa. This characteristic was unfortunately carried over to a considerable extent into the cultivated species. *G. herbaceum* is also difficult to deal with under local conditions, and for this reason and also on account of its low content of recessive genes, the greater part of the backcrossing was confined to *arboreum*.

A large number of different strains of *arboreum* and *herbaceum* were used in this investigation. They are listed below in order of their type numbers:

A 1	<i>C.W.*</i> ; Cawnpore white flower, $y_a$	India
A 7	" <i>G. cernuum</i> ," pale flower, $Y_a^P$	Bengal-Assam
A 8	<i>B.L.</i> ; Burma laciniated	Burma
A 15	Pale flower, $Y_a^P$	Burma
A 16	Ghost spot, $R_2^{OS}$	China
N 5	<i>B.G.</i> , pale flower, $Y_a^P$ ; ghost spot, $R_2^{OS}$	Burma
N 9	<i>A.H.</i> ; carries $Cp_a$ (crumpling)	Sudan
N 14	White flower, $y_a$ ; ghost spot, $R_2^{OS}$	India
N 19	Wagale lintless, $h_a$	Burma
N 24	Petalody heterozygote, $Pdy\ pdy$	South India
N 25	Pale pollen, $p_a$	South India
N 44	Chinese pale flower, $Y_b^P$	China
H 10	Spotless, $R_2^{AO}$	Afghanistan
H 16	Cream pollen, $p_a$	Tashkent
O 1	1027 ALF	Surat, India
O 7	Bushveld cotton	Portuguese East Africa
O 8		Transcaucasia

\* In the case of a few strains reference by these symbols has been made in previous publications from this Station.

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Following the recent revision of the classification of the Asiatic cottons by Hutchinson & Ghose (1937*a*), these strains belong to the taxonomic groups indicated below. The two species were subdivided by these authors primarily on the basis of the perennial or annual habit into varieties, and then by distribution into forms. Some of the more important genetic differences however appear to be associated rather with geographic distribution (Silow, 1939*b*) than with the acquisition of the annual habit, and for this reason it will be more satisfactory when it is necessary to discuss in the text the taxonomic ranking of particular strains, to refer briefly to their formal status only:

<i>G. arboreum</i> var. <i>typicum</i> forma <i>soudanensis</i>	N 9
<i>G. arboreum</i> var. <i>neglectum</i> forma <i>indica</i>	N 24, N 25
<i>G. arboreum</i> var. <i>neglectum</i> forma <i>bengalensis</i>	A 1, N 14
<i>G. arboreum</i> var. <i>neglectum</i> forma <i>burmanica</i>	A 8, A 15, A 16, N 5, N 19, N 44
<i>G. arboreum</i> var. <i>cernuum</i>	A 7
<i>G. herbaceum</i> var. <i>typicum</i>	H 10, H 16
<i>G. herbaceum</i> var. <i>frutescens</i>	O 1, O 8
<i>G. herbaceum</i> var. <i>africanum</i>	O 7

The following synthesized multiple recessive types were also used for particular backcrosses:

- T 1 Multiple recessive lintless selection, predominantly *arboreum*, with trace of *herbaceum* in ancestry
- T 3 Multiple recessive cream pollen selection, from  $F_2$  of H 16  $\times$  T 14
- T 4 Multiple recessive pale pollen selection, from  $F_2$  of N 14  $\times$  N 25
- T 6 Multiple recessive linted  $h_a$  selection; same origin as T 1
- T 14 Multiple recessive selection, from  $F_2$  of A 1  $\times$  N 5 (130*d*)
- T 17 Multiple recessive petalodic selection, from  $F_2$  of N 14  $\times$  N 24

All pollinations were performed in insect-proof greenhouses after emasculation. In most cases it was more convenient as a routine procedure to use hybrid material as the seed parent, though reciprocal crosses were frequently performed and their progeny analysed separately.

In an investigation of this nature it is not possible to plan a comprehensive series of tests right from the start, as it is not known beforehand what will segregate. In the early stages especially segregating families must be used as they appear to demonstrate the nature of the parental type. Rather than describe each of the hybrids and their progeny in turn it has therefore been considered more suitable to discuss independently each of the main characters studied, bringing together all related information from various sources. The gene symbols used are based on the revised list of Hutchinson & Silow (1939).

## II. COMPATIBILITY AND FERTILITY

*G. anomalum* has been crossed with representatives of the chief subdivisions of the genus, and it will be worth summarizing the available information on its behaviour.

(1) *With New World wild diploid species.* Skovsted (1935, 1937) has reported that *anomalum* crosses easily with *aridum* (Rose & Standley) Skovsted, *dauidsonii* Kellogg, and *thurberi* Tod., but both he and Webber (1939) found that the hybrids were completely sterile, and their conjugation was as low as 0.2–5.7 bivalents ( $2n=26$ ). Skovsted found that when *anomalum* is crossed with *armourianum* Kearney or *sturtii* F.v.M. bolls set freely, but contain only empty seeds. Similar behaviour with *raimondii* Ulb. has been reported by Hutchinson (1939).

(2) *With New World non-cultivated tetraploid species.* Skovsted (1937) reported that *anomalum* crosses relatively easily with *tailense* Parl., but most of the seeds obtained were empty or contained only partially developed embryos. One unthrifty hybrid was grown. With *darwinii* Watt *anomalum* crossed less easily, giving only a few empty seeds.

(3) *With New World cultivated tetraploid species.* Skovsted (1937) found that *anomalum* crossed with some difficulty with *barbadense* L., giving mostly empty seeds. Compatibility with *hirsutum* L. was not adequately tested. Further information has been collected, and this together with that reported by Skovsted (1937) may be summarized as follows:

♀	♂	Flowers pollinated	Capsules set	Total seeds	Empty seeds	Hybrids raised
<i>anomalum</i>	<i>barbadense</i>	84	26	120	All	—
<i>anomalum</i>	<i>hirsutum</i>	46	7	32	All	—
<i>barbadense</i>	<i>anomalum</i>	313	19	49	All but 4	1
<i>barbadense</i> haploid	<i>anomalum</i>	208	2	2	1	1
<i>hirsutum</i>	Mixed*	284	44	142*	None	5*
G 9543	<i>anomalum</i>	26	6	42	None	30

\* A little own or other compatible pollen carrying a marker gene was mixed with that of *anomalum* to improve setting. Only five of the progeny were the result of the interspecific cross.

When *anomalum* was used as female all seeds obtained were empty. With *barbadense* as female relatively fewer seeds set than in the reciprocal cross, but about 10% of them contained fully developed embryos. The *barbadense* strain used was St Vincent Superfine, V 135. With *hirsutum* as female the seed setting was proportionally even lower, although a little compatible pollen had been mixed with that of *anomalum* to improve boll setting, but all seeds which were the result of the interspecific cross contained normal embryos. The cross-compatibility of plant G 9543 was of interest in this connexion. This plant was one of the selfed progeny of a sixth backcross of *hirsutum* to the same *barbadense* strain as used above (V 135), and therefore predominantly of the latter genotype, yet considerably more compatible with *anomalum* than the pure strain, and all seeds, instead of only 10%, contained normal embryos. Evidently in

such cases as these, which are on the borderline between compatibility and incompatibility, slight differences in constitution may considerably modify crossing behaviour. All the *anomalum*-New World hybrids raised have been completely sterile to their own, New World, *anomalum* and Asiatic pollen. Skovsted & Webber found a mean pairing of only 2.6-10.48 in these hybrids with  $2n=39$ .

(4) *With wild Asiatic diploid species.* Skovsted (1935) reported that Hutchinson had raised four hybrids of *anomalum*  $\times$  *stocksii* M.Mast., which died after producing only a few leaves.

(5) *With cultivated Asiatic diploid species.* The majority of *anomalum* flowers crossed in either direction with *arboreum* L. and *herbaceum* L. set bolls which contain a full complement of seeds with fully viable embryos. Skovsted & Webber have found that the mean pairing in these hybrids with  $2n=26$  is as high as 10-12; the former author found that approximately 25% of pollen mother cells examined showed complete pairing. Webber found this figure to be as high as 50%, and also stated, without citing any data, that the hybrid between *anomalum* and *sanguineum* (a variety of *arboreum*) is 45% fertile when selfed or back-crossed. Experience at this Station would not, however, lead to the assessment of fertility as anything like as high as this. Actually it is not easy to state fertility figures in simple terms for cotton, as bolls containing a low proportion of pollinated ovules frequently drop before maturity, quite apart from the fact that setting is very sensitive to slight physiological derangements totally distinct from compatibility. Both the percentage of flowers which set capsules, and the proportion of ovules which set seed within these capsules should be taken into consideration. Such data with reference to three *arboreum* and four *herbaceum* hybrids are summarized in Table 1. The full complement of seeds per capsule is about 20 in the *arboreum* and *herbaceum* types, except in the case of *herbaceum* H 10 which usually contains about 32-36 seeds. *G. anomalum* has a complement of about 10-12 seeds. Capsules on all of the hybrid plants contained about 20 ovules. Cotton flowers normally become pollinated autonomously. These hybrids must have borne at least 6000 flowers in the greenhouse, but not a single boll set. To a very slight degree this is due to the fact that their poor pollen does not readily become transferred to their unusually long stigmas, but the selfing data in Table 1, referring to flowers in which pollen was transferred by brush from anthers to stigma, indicates that in any case functional gametes are exceedingly few. In the *arboreum* hybrids approximately only 10% of self-pollinated flowers set bolls, containing only 10-20% of their full

complement of seeds. The *herbaceum* hybrids appear to be of even lower fertility, though this may partly be due to the fact that *herbaceum* types and their hybrids tend to shed very readily for purely physiological reasons under local conditions. In the few bolls which matured the proportion of ovules fertilized was much the same as in *arboreum* hybrids. On backcrossing the hybrids as female (after emasculation) with Asiatic types, fertility was higher. About 50% of the flowers pollinated set, and these capsules contained about 25% of their complement. Boll setting was again lower in *herbaceum* than in *arboreum* hybrids, but the proportion of seeds set per boll much the same. The reciprocal pollinations, in which the hybrids were used as male parents, showed a similar

Table 1. *Fertility of anomalum-Asiatic hybrids on selfing and backcrossing to Asiatic types*

Hybrid	Backcross to Asiatic types								
	Selfing			Hybrid as ♀			Asiatic as ♀		
	Flowers pollinated	% capsules set	Seeds per capsule	Flowers pollinated	% capsules set	Seeds per capsule	Flowers pollinated	% capsules set	Seeds per capsule
<i>anomalum</i> × <i>arboreum</i> A 8	150	7	4.1	816	54	5.5	12	(0)	.
<i>anomalum</i> × <i>arboreum</i> A 16	48	8	3.0	318	47	5.6	4	(25)	5.0
<i>anomalum</i> × <i>arboreum</i> N 14	92	13	2.0	109	68	5.1	39	59	7.0
<i>anomalum</i> × <i>herbaceum</i> H 10	128	0	.	360	22	7.5	77	25	5.2
<i>anomalum</i> × <i>herbaceum</i> O 1	82	0	.	4	(0)	.	26	8	7.5
<i>anomalum</i> × <i>herbaceum</i> O 7	358	1	2.7	14	(0)	.	38	.	.
<i>anomalum</i> × <i>herbaceum</i> O 8	209	0	.	86	12	2.4	38	16	7.0

Unreliable percentage estimates based on low numbers are in brackets.

degree of setting. Actually it might have been expected that with the heavy pollinations used there would have been an excess of functional male gametes over the number required to give a full set of seeds on the pure Asiatic seed parents, but this was not so. A further complication in the way of stating fertility of these hybrids in simple terms is introduced at this stage, since many of the seeds obtained contained only imperfectly developed embryos or were quite empty. Some 1500 seeds derived from the hybrid (*anomalum* × *arboreum* A 8) pollinated by *arboreum* N 14 or *herbaceum* H 10 were carefully examined, and usually only 75–85% were fully developed, and in some batches pollinated at different times or from different sib plants, this figure was as low as 50%. Bearing in mind the proportion of flowers which set bolls, the proportion

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of seeds within the bolls which set, and the proportion of those seeds which contained good embryos, it may be estimated that the fertility of hybrids between *anomalum* and *arboreum* or *herbaceum* is no greater than 10% on backcrossing, whilst on selfing it is as low as 1%. These figures of course refer to fertility in terms of potential seed-setting capacity and, in view of the multiplicity of factors operating, are not exact estimates of the proportion of functional gametes.

The hybrids have also been backcrossed to a small extent to the *anomalum* parent. The figures are not sufficiently extensive to warrant citation, but the indications are that their fertility with *anomalum* is of comparable degree with that with the cultivated Asiatic species.

Only a very limited number of  $F_2$  progeny of these hybrids have been grown. Not only did a high proportion of the very few seedlings

Table 2. *Ovule fertility of backcrosses to arboreum and herbaceum*

	Mean seeds per boll*														
	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28
Plants of the 1st backcross to <i>arboreum</i>	Several	.	1	2	7	1	.	.	.	.	.	.	.	.	.
Plants of the 2nd backcross to <i>arboreum</i>	1	.	1	1	.	2	1	1	1	.	1	.	.	.	.
Plants of the 3rd backcross to <i>arboreum</i>	.	.	.	.	.	.	.	4	1	1	.	.	.	.	.
Plants of the 1st backcross to <i>herbaceum</i>	Several	.	.	.	.	.	1	1	.	.	.	.	.	.	.
Plants of the 2nd backcross to <i>herbaceum</i>	1	.	.	.	.	.	.	.	.	1	3	.	1	.	1

\* 20 represents a full set in *arboreum* backcrosses; the *herbaceum* type used for these backcrosses contained up to 28 or 36 seeds per boll.

obtained die early or fail to mature, but in addition the extremely wide modifier segregation precluded the possibility of any useful genetic analysis. Even in the first backcross, with its considerably lower genetic variance, interpretation was in most cases impossible.

The high fertility which was rapidly attained on recurrent backcrossing into the cultivated species is indicated in Table 2. This shows, in the form of a frequency table, the fertility of certain plants backcrossed for genetic purposes in the first backcross and subsequent generations. This table does not indicate segregation for fertility in any way, but only indicates the approach to fertility which was attained. It is not a random sample of the populations since conscious selection was practised in propagating only plants which were setting in the field or showed reasonably good pollen. Only the seed content of the bolls which set is indicated; as fertility is approached this affords a better impression of

fertility than the percentage of flowers which set, as even on a fully fertile non-hybrid plant a full set of capsules is rarely attained, for physiological reasons as already mentioned.

In the first backcross to *arboreum* or *herbaceum* some plants were completely sterile. Dr Skovsted, formerly of this laboratory, examined somatic plates of one group of 49 plants of the backcross (*an.*  $\times$  A 8)  $\times$  N 14, and found that 46 of these had the normal diploid number of chromosomes, whilst the remaining three had  $2n=27$ . The latter plants were quite sterile; one of them was very stunted. All of the others, which were backcrossed, set seed to some extent. The plants included in the frequency array in Table 2 were members of another series which were more extensively backcrossed. Up to 89% of the flowers pollinated set on one of the first backcross plants; others set a lower proportion of flowers. Of the plants in the first backcross to *arboreum* which set, it will be seen from Table 2 that most contained about eight seeds per capsule, which is nearly twice the number set on the  $F_1$ , and represents about 40% of the seed complement. In the second backcross there were still some sterile plants present, but on the whole the mean fertility of plants as shown by the seed content of their bolls was higher, and even in this generation some contained the full complement of 20 seeds. The average fertility in the third backcross was even higher, and fewer sterile plants appeared. The same course of increasing fertility is evident from the two backcross generations to *herbaceum* which are shown.

Fourth and fifth backcross progenies have also been grown, and selfed progenies of these later backcrosses. Since most of these are of complex constitution, having been backcrossed to either of the cultivated species at different stages for particular genetic tests, it is not worth citing fertility figures. It is important to note that high fertility can eventually be attained as a result of recurrent backcrossing, whilst retaining at the same time at least one or two genes of *anomalum* origin. In practically all selfed progenies from late backcrosses which have been grown a very slight degree of infertility has been observed—for instance, the occasional appearance of petalody of stamens. In this connexion it must be stated that the primary object of this investigation was genetic; stringent selection for fertility was not practised, but the results obtained suggest that if it had been, absolute fertility could have been attained along with the transference of particular genes from the wild to the cultivated species.

With reference to the compatibility of other wild species with the cultivated Asiatics, Skovsted (1935) reported only empty seeds on



crossing with *armourianum* and *davidsonii*, and a great preponderance of empty seeds on crossing with *sturtii*, though in this case two sterile hybrids were also obtained. Hutchinson (1939) also obtained only empty seeds on crossing with *raimondii*. With *aridum*, *thurberi*, and *stocksii*, Skovsted (1935) and earlier workers obtained only completely sterile hybrids. Thus no wild species other than *anomalum* has given any fertility in hybrids with the cultivated Asiatic cottons. Since no other hybrids involving *anomalum* which have been produced have given any fertility either, the interest attaching to the *anomalum*-Asiatic crosses is readily apparent.

### III. COROLLA COLOUR

#### (1) *The situation in the cultivated Asiatic species*

Hutchinson (1931) has shown that the three main corolla colour types encountered in *G. arboreum*, full yellow, pale yellow, and white, are due to a multiple allelomorph series which he designated Y-Yp-y. He confirmed earlier indications of a physiological relationship between corolla colour and size, yellow flowers having long petals and whites short, with pales of intermediate length. For the classification of segregating progenies he made use of a graded series of paintings, in which the deepest yellow was numbered 10, and white was 1. On this scale full yellow types carrying Y grade from 7 to 10, and y types are grade 1. Pale Yp types are very much nearer white than yellow in intensity of colour, usually grading at 2, very occasionally at 1.5 or 3. As far as length is concerned the relationship is the reverse; Hutchinson's petal length estimates within segregating progenies showed white petals about 20-25% shorter than full yellows; but pales were very nearly the same length as full yellows, being only 1-2% shorter, and the difference barely significant. Hutchinson also showed that there are modifiers affecting intensity of corolla grade, and that these affect petal length in the same way as the main genes. Thus in *G. herbaceum*, which almost invariably carries Y, many yellow-flowered strains are both paler and shorter than those in *arboreum*.

A pale yellow-flowered strain of *arboreum* from China, grown under the type number N 44, has recently been studied by the writer. It is very similar to types carrying Yp, but is distinguishable in that whilst the greater part of the lamina of the petal is pale yellow of grade 2, there is a very slight intensification of yellow towards the base of the petal round the margin of the anthocyanin spot, a tendency absent from the more common type of pale. The N 44 strain is complementary with

common pale and white-flowered strains, giving the ordinary full yellow. This demonstration of complementary factors necessitates the addition of a subscript to the symbols used by Hutchinson, and in order to bring the system of nomenclature into conformity with accepted genetic convention, the pale allele should be indicated by a superscript. It is therefore proposed that the series described by Hutchinson, that most frequently encountered in *arboreum*, be designated  $Y_a-Y_a^P-y_a$ , and the pale concerned in the Chinese strain  $Y_b^P$ . On analogy with the  $Y_a$  series it seems quite possible that a lower allele may be encountered in the future, and the suggested allocation makes allowance for this eventuality. On this scheme ordinary yellows are regarded as  $Y_a Y_b$ , the common pales and whites as  $Y_a^P Y_b$  and  $y_a Y_b$  respectively, and the Chinese pale strain as  $Y_a Y_b^P$ .

In this investigation it was not found that distinctions between Hutchinson's grades above 7 could be made with any confidence, though no difficulty was found in using the lower grades. It was therefore decided to grade up to class 8 only, reserving this for the most intense yellows. The vast majority of full yellows were graded at 7. The grades are not reproduced here, but may be seen in Hutchinson's 1931 paper. Flowers were collected from the field in the early morning, and graded in the laboratory; three readings were taken from flowers collected on different days. For the estimation of flower size, the length of the longest petal on three flowers was measured from the point of insertion to the extreme tip.

## (2) *Hybrids and first backcross progeny*

*G. anomalum* has a very pale cream, almost white corolla, with a faint tinge of pink (Pl. 18, fig. 4). The flower is of medium size, with petals 30–37 mm. long. It grades approximately at 2, though exact comparison with the scale of yellows is difficult on account of the pink tinge. It will be shown later that this pink is associated with one of the main anthocyanin spot genes, but in this section it is proposed to deal with the inheritance of the yellow flavone only, ignoring as far as possible the complications in corolla colour introduced by the anthocyanin.

*G. anomalum* was crossed with full yellow, pale and white representatives of *arboreum* and *herbaceum*. In each  $F_1$  from two to twenty plants were examined, and the results of their corolla colour grading are shown in Table 3. Each progeny was quite uniform within itself, but scoring on the individual plants varied slightly from day to day as shown. Grading of yellow intensity was not easy on account of the

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intensification of the accompanying pink, especially in the hybrids involving full yellow Asiatic types. The interaction between flavone and anthocyanin will be discussed later. At this stage it is only necessary to state that the development of petal red was later found to be dependent to some extent upon exposure of the bud to sunlight, and it is to differences in this exposure that a great deal of the observed variation in pink, and consequently in yellow intensity, can be attributed. Quite apart from this physiological interaction, the variation in pink naturally introduced a serious subjective error in yellow estimation. The same diffi-

Table 3. *Yellow corolla grade in  $F_1$  hybrids. G. anomalum = grade 2*

Type no.	Asiatic parent		$F_1$
	Gene	Grade	Grade
A 8	$Y_a$	7	4-5 (see Pl. 18, figs. 1, 5)
A 16	"	7-8	3-4 (see Pl. 18, figs. 3, 7)
N 25	"	7-8	5
H 10	"	7	3-5 (see Pl. 18, figs. 2, 6)
H 16	"	5	5-6
O 1	"	7-8	3-5
O 7	"	7	4-5
O 8	"	8	4-5
N 5	$Y_a^P$	2	4-6
N 14	$y_a$	1	4-5
T 14	"	1	4-5
N 44	$Y_b^P$	2	3-4

culties were encountered in grading the first backcrosses, data for which are summarized in Table 4. The main findings requiring interpretation were as follows:

(i) The  $F_1$  hybrids were all of approximately the same shade of low-grade yellow, no matter whether the Asiatic parent were full yellow, pale, or white (Table 3).

(ii) In backcrosses to Asiatic full yellow there was a very wide modifier segregation, and full yellows reappeared (Table 4, *a*). The modifier segregation was much wider than is encountered in crosses between *arboreum* and *herbaceum*. The two most nearly comparable cases available are Hutchinson's (*arboreum* pale  $\times$  *herbaceum* full yellow) backcrossed to *herbaceum* full yellow, shown in his 1931 Table 1, and the (*anomalum* pale  $\times$  *arboreum* full yellow A 8) backcrossed to *herbaceum* full yellow H 10, shown in the first line of Table 4:

	Yellow grades						Total
	8	7	6	5	4	3	
( <i>arb. pale</i> $\times$ <i>herb. yellow</i> ) $\times$ <i>herb. yellow</i>	.	1	29	102	59	.	191
( <i>an. pale</i> $\times$ <i>arb. yellow</i> ) $\times$ <i>herb. yellow</i>	71	116	107	101	97	58	550

Table 4. Segregation for yellow corolla grade in first backcrosses

	Yellow grades											Total	
	8	7	6	5	4	3	2.5	2	1.5	1		Full yellow	Pale yellow or white
(a) <i>anomalum</i> × <i>full yellow</i> , backcrossed to <i>full yellow</i> : ( <i>an.</i> × A 8) × H 10 ( <i>an.</i> × A 8) × A 8 ( <i>an.</i> × A 16) × A 16	71 2 31	116 7 48	107 7 5	101 2 11	97 8 13	58 5 .	.	.	.	.	.	550 31 108	.
(b) <i>anomalum</i> × <i>full yellow</i> , backcrossed to <i>anomalum</i> : ( <i>an.</i> × A 16) × <i>an.</i> ( <i>an.</i> × H 10) × <i>an.</i>	.	.	2 1	2 6	1 3	.	6 .	.	.	.	.	5 12	6 11
(c) <i>anomalum</i> × <i>full yellow</i> , backcrossed to <i>arboreum white</i> : ( <i>an.</i> × A 8) × N 14 ( <i>an.</i> × H 10) × N 14 or T 14	7 4	37 10	26 11	37 10	44 11	49 1	.	.	.	.	.	200 47	.
(d) <i>anomalum</i> × <i>pale</i> , backcrossed to <i>arboreum pale or white</i> : ( <i>an.</i> × N 5) × N 14, white ( <i>an.</i> × N 5) × N 5, pale	.	4 1	.	.	.	.	.	1 2	3 .	.	.	4 5	4 3
(e) <i>anomalum</i> × <i>white</i> , backcrossed to <i>arboreum pale or white</i> : ( <i>an.</i> × N 14) × N 14, white ( <i>an.</i> × N 14) × N 5, pale*	12 .	26 36	25 .	9 14	12 .	.	.	.	.	88 49	.	84 50	88 49

\* Progeny not graded in this backcross, but grouped into three classes (i) Y 8-7-6; (ii) Y 5-4; (iii) Y<sup>P</sup> 2-1.5-1. (i) and (ii) not distinct from each other; (iii) quite distinct.

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The much greater genetic variance in the *anomalum*  $\times$  *arboreum* cross than in the *arboreum*  $\times$  *herbaceum* cross is clearly evident in this comparison. This particular cross of Hutchinson's shows the greatest amount of modifier segregation which he encountered in the inter-species crosses within the cultivated Asiatics which he studied.

(iii) *Anomalum*  $\times$  full yellow backcrosses to *anomalum* (Table 4, *b*) indicated a single main factor difference between the parents.

(iv) The  $F_1$  of *anomalum*  $\times$  full yellow, when backcrossed to *arboreum* white (Table 4, *c*), gave no progeny of appreciably lower grade of yellow than the  $F_1$ , though in the other direction full yellows were again recovered. The absence of pale segregates in these backcrosses indicated that *anomalum* pale and *arboreum* white are not homologous. The evidence, together with that in (i) above, suggests that *anomalum* and pale or white *arboreums* carry complementary factors for yellow corolla. The appearance of full yellows in these backcrosses and in those discussed in (ii) above, and the uniform low grade of all the  $F_1$  hybrids, suggested the presence of one or more yellow depressors in *anomalum*.

(v) The backcrosses from *anomalum*  $\times$  *arboreum* pale or white to the latter types (Table 4, *d*, *e*) showed a single main factor difference between these parental types.

### (3) *A third main locus, Y<sub>c</sub>*

The hypothesis that *anomalum* is complementary with pale and white corolla types of *arboreum*, and that it carries one or more yellow depressors, received confirmation from certain segregates in a series of backcrosses to A 8 of the hybrid *an.*  $\times$  A 8 full yellow. The wide segregation in the first backcross has been shown in Table 4, *a*. Very small second backcross progenies from two first backcross selections of grade 6 were grown, primarily for transference of the leaf-shape gene from the wild species, and in these corolla colour was not scored. From some of the second backcross plants third backcross and selfed progenies were grown in the following season. By that time this material had been superseded for the original purpose of leaf shape transference by other lines and was only planted in the field at the end of the season after more important material had received attention. On that account mortality was high, and only a few plants survived. Amongst these it was noticed that, in addition to full yellows of grades 8-7-6, there were some very clear-cut segregates of grade 4 (fluctuating to grade 5) in one of the backcrosses, and of grade 3 in one of the selfings (Table 5). These two groups of lower grade segregates were actually much more distinct from each

other than would appear from their gradings. As a working hypothesis it was thought that the grade 3 plant (no. 14,868) in the selfed progeny carried the transferred *anomalum* corolla colour gene extracted as a homozygote, and that the grade 4 plants (nos. 14,859 and 14,862) in the backcross progenies might be heterozygotes for this same gene. Accordingly the former plant was selected, and both of the latter and their sibs and some of their half-sibs. The behaviour of selfed progenies from these selections (Table 5, third backcross selfed column) effectively disposed of this hypothesis, since the grade 4 parents gave only grade 8-7 and grade 4 progeny, whilst the grade 3 type appeared in the progeny of

Table 5. *Corolla grade segregation in third backcross of (an.  $\times$  A 8) to A 8 full yellow, and in selfed progenies of second and third backcross selections*

1st B.C. plant nos.	2nd B.C. plant nos.	3rd B.C. yellow grades				2nd B.C. selfed yellow grades		3rd B.C. selfed yellow grades		
		8	7	6	4	7	3	8-7	5-4	3
P 1169	P 1874	.	1	.	.	2	1 <i>a</i>	.	.	.
	P 1875	2	.	1	.	1	.	.	.	.
	P 1876	3	2	.	.	.	.	.	.	.
P 1170	P 1877	5	.	.	.	.	.	28	.	7 <i>b</i>
		.	.	.	.	.	.	69	.	21
		.	.	.	.	.	.	11	.	.
		.	.	.	.	.	.	16	.	.
	P 1881	3	.	.	2 <i>c</i>	.	.	81	.	<i>d</i>
		.	.	.	.	.	.	34	.	<i>d</i>
		.	.	.	.	.	.	41	.	<i>d</i>
		.	.	.	.	.	.	35	49	<i>e</i>
		.	.	.	.	.	.	6	11	<i>e</i>

*a* This grade 3 segregate was no. 14,868.

*b* One of these grade 3 segregates was no. 9992.

*c* These grade 4 segregates were nos. 14,859 and 14,862.

*d* Parents of these progenies were grade 8.

*e* Parents of these progenies were grade 4 (nos. 14,859 and 14,862).

some of the full yellow selections. Furthermore, as will be discussed later, no grade 3 segregates appeared in the progeny of 14,868 when crossed with 14,859 and 14,862.

The breeding behaviour of the grade 3 type will be discussed first, but before doing so it is necessary to emphasize that it must not be presumed that it bears any genotypic relationship to the grade 3 phenotype which appeared in the first backcrosses. First, the modifier *welter* in the latter should preclude any such assumption. Secondly, plants of the grade 3 type in the first backcross all showed a strong development of pink, and it will be demonstrated later that interaction between flavone and anthocyanin may lead to the lowering of grade of yellow. Actually

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the early backcross grade 3 types correspond to the grade 4 type here under study, from which complications due to anthocyanin were absent. The extracted grade 3 type appeared only in selfed progenies, and these particular lines had relatively uniform modifier backgrounds. Although pink was present in some of these lines, it was not expressed in the grade 3 segregates.

The original grade 3 segregate, no. 14,868, which appeared in the selfed second backcross family, unfortunately died before any selfed seed could be obtained from it, but as it gave rise to some outcross progeny which will have to be discussed, it is necessary to establish its constitution. It was indicated, in connexion with Table 5, that plants of this phenotype reappeared in the following season in some selfed progenies of third backcross full yellow selections. It will be seen from Table 5 that these and 14,868 trace back to different first backcross plants. That they were of the same genotype as 14,868 is shown by the fact that only grade 3 progeny appeared in crosses between one of them (no. 9992), and certain double recessives extracted from an  $F_2$  involving 14,868 and the *arboreum* pale petal type N 5. This point will be discussed in more detail below, but is important here in indicating that both 14,868 and 9992 may be considered as being of the same constitution.

In the two third backcross selfed families which threw grade 3, the ratios 28 : 7 and 69 : 21 were indicative of a monofactorial 3 : 1 segregation. The grade 3 selection when selfed gave 14 progeny, all grade 3. Either 14,868 or 9992 was crossed with full yellow, pale yellow  $Y_a^P$ , white  $y_a$ , and Chinese pale  $Y_b^P$  representatives of *arboreum*. All progeny in each case were full yellow of grade 8-7:

Cross	No. of progeny observed
14,868 $\times$ A 8, full yellow	9
9992 $\times$ A 16, full yellow	5
14,868 $\times$ A 7, pale yellow $Y_a^P$	3
14,868 $\times$ N 5, pale yellow $Y_a^P$	11
9992 $\times$ N 5, pale yellow $Y_a^P$	8
14,868 $\times$ N 14, white $y_a$	15
9992 $\times$ N 44, Chinese pale $Y_b^P$	10

This extracted grade 3 behaved as a complementary type with the *arboreum* recessive corolla colour types in exactly the same way as the original *anomalum* did, except that the latter only gave low grade yellow hybrids on account of the presence of a yellow depressor, as will be evident later.

Two plants from the cross 14,868  $\times$  N 5 were backcrossed to *anomalum*, and gave small progenies containing in all 8 plants of low grade yellow

similar to the original *anomalum*-Asiatic hybrids, and 14 plants of exactly the same corolla colour as the wild species itself. This shows that the extracted grade 3 type contains the main pale corolla colour gene derived from *anomalum*. The higher grade of the transferred pale is due to the loss of the yellow depressor. At this stage it will be a convenience for discussion to assign the symbol  $Y_c^P$  to this pale corolla gene.

Five  $F_1$  hybrids between pale 9992 and full yellow A 16, when back-crossed to 9992, gave clear-cut segregation into grade 7 and grade 3 as follows:

Plant no.	Grade 7	Grade 3	$\chi^2_{1:1}$	P
P 4098	38	16	9.0	.
P 4126	52	30	5.9	.
P 4127	40	32	0.9	.
P 4128	45	35	1.2	.
P 4129	37	36	0.0	.
Total	212	149	11.0	Very small
Heterogeneity	.	.	6.0	0.2

These five families were homogeneous amongst themselves, but gave in all a significant deficiency of recessives on a 1 : 1 basis. Introduced genes were frequently found to be in defect in this study, and this problem will be discussed later. From all the other evidence on corolla colour which has been obtained it appears reasonable to interpret this ratio as a distorted 1 : 1, indicating that the grade 3 type differs from full yellow in one gene.

Two of the full yellow  $F_1$  hybrids between pale 14,868 ( $Y_a Y_c^P$ ) and pale N 5 ( $Y_a^P Y_c$ ) gave a remarkably clear complementary factor ratio on selfing. Since both A 8, to which the *anomalum* pale had been transferred, and N 5 are Burmese forma *burmanica* representatives, the absence of any marked modifier segregation was not surprising. Four main classes were observed:

- (a) Full yellows, ranging 8-7.
- (b) Pale yellows of grade 3.
- (c) Pale yellows of grade 2.
- (d) Very pale yellows, almost white, grade 1+.

After becoming familiar with the type of segregation, scoring of individual plants from day to day was very consistent, and all plants could be assigned without difficulty to one of these four classes, except a very small proportion of the pales which were intermediate between grades 2 and 3. Although no further genotypic testing was performed, except in the case of the class (d), the four groups may be taken as



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corresponding to the four expected genotypes  $Y_a Y_c$ ,  $Y_a Y_c^P$ ,  $Y_a^P Y_c$ ,  $Y_a^P Y_c^P$  on the following grounds:

(1) Class (a) was perfectly distinct from the other three pale yellow classes.

(2) The *anomalum* pale is far more distinctive phenotypically from the common Asiatic pale than the classification into grades 3 and 2 indicates. Part of this distinction is associated with the fact that the  $Y_c^P$  type is considerably shorter than full yellow or  $Y_a^P$  types. Petal length measurements were not taken in this family, but this general observation is confirmed by measurement in the  $Y_b^P$  cross which will be discussed next.

(3) The constitution of the near-white class (d) was confirmed genetically as shown below. On this basis the segregation may be taken as conforming to a 9 : 3 : 3 : 1 as follows:

Family	Yellow grade					Total
	8-7	3	2-5	2	1+	
3046	55	11	1	12	3	82
3047	45	14	2	10	3	74
Total	100	25	3	22	6	156
Expected 9 : 3 : 3 : 1	87.75	29.25		29.25	9.75	156

The families are sufficiently similar to warrant combination, and the totals accord well with expectation ( $\chi^2=4.5$ ,  $P=0.2$ ). Two selections from the grade 1+ group, supposed double recessives, were each crossed with both of their parental types 9992 and N 5, and all progeny were pales of grade 3-2. The number of progeny examined in each cross was as follows:

	× 9992	× N 5
Grade 1+ selection no. 15,723	3	6
15,759	4	16

That these selections were not complementary with either of their parental pales confirms the supposition that they were double recessives.

Since the phenomena of complementary alleles at the same locus and of complementary recessive genes in duplicate loci will be demonstrated in connexion with anthocyanin inheritance, it is necessary to indicate that the more normal interpretation of complementary factors applies to this case of corolla colour. If it were a case of complementary alleles at the same locus, the  $F_2$  would have given an allelic 2 : 1 : 1 ratio, which could easily be confused with a 9 : 3 : 3 : 1 if the latter term were incorporated with either of the middle terms giving a 9 : 3 : 4. However, the demonstration of the presence of pale segregates giving no comple-

mentary effect with either parental pale disposes of the possibility that the latter might be due to complementary alleles.

A third plant from the  $F_1$  between 14,868 and N 5, when backcrossed to N 5, segregated 31 full yellows and 33 pales of the N 5 type, in conformity with expectation.

Similar complementary factor ratios were observed in two  $F_2$  families derived from the full yellow hybrids between pale 9992 ( $Y_b Y_c^P$ ) and N 44 Chinese pale ( $Y_b^P Y_c$ )—again forma *burmanica*. There was a satisfactory distinction between full yellows, the grade 3 *anomalum* pales with short petals, the “grade 2 Chinese” type with the characteristic slight intensification of yellow just around the red petal spot, and with petals of practically the same length as the full yellows, and a fourth class with very pale, practically white, corolla showing a very slight intensification of yellow near the spot, and with petals of intermediate length—presumably the double recessives ( $Y_b^P Y_c^P$ ).

Family	Yellow grades					Total
	8-7	4	3	2 Ch	1 + Ch	
3715	34	.	5	13	3	55
3716	33	1	3	4	3	44
Total	67	9		17	6	99
Expected 9 : 3 : 3 : 1	55.6	18.6		18.6	6.2	99.0

Agreement with expectation was not quite as good as in the previous cross considered ( $\chi^2=7.5$ ,  $P=0.1-0.05$ ), but most of the discrepancy was due to a deficiency of *anomalum* pale segregates. Nevertheless, it is clear that *anomalum* pale is independent of Chinese pale, as well as of the common Asiatic pale or white locus.

#### (4) *A yellow depressor*, *Ydp*

It has already been mentioned, at the beginning of the preceding section, that two grade 4 segregates (nos. 14,859 and 14,862) were observed in the third backcross of *anomalum* to full yellow A 8, and their segregation on selfing was summarized in Table 5. It was thought at first that they might be heterozygous for the grade 3 *anomalum* pale type, but quite apart from the fact that heterozygotes for the latter were later found to be full yellow, 108 progeny derived from their backcross with *anomalum* itself were all yellows of grade 4-5. Furthermore they threw nothing paler than themselves either on selfing or crossing to the grade 3 plant no. 14,868. In each case they gave two reasonably distinct groups, one fluctuating between 7 and 8, and the other from just below grade 4 to just above grade 5. Within these groups it was not felt that

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classification was reliable, but there was no hesitation in classifying them into the full yellow and the "depressed yellow" class, as it was termed in order to differentiate from true pales. The fact that none of the latter appeared indicates that 14,859 and 14,862 were both of  $Y_c Y_c$  constitution. These two plants were also crossed with white petalled N 14 ( $y_a$ ) and gave two similar clear-cut phenotypes. In all, the segregations from these two plants were as follows:

Progeny	Full yellow	Depressed yellow
14,859 selfed	35	49
14,862 selfed	6	11
14,859 $\times$ 14,862	12	12
14,862 $\times$ 14,868	12	6
14,859 $\times$ N 14	95	101
14,862 $\times$ N 14	109	88

The backcross ratios were very suggestive of the presence of a single dominant depressor, but the two selfings did not conform at all well to the 3 depressed : 1 full yellow expected on that basis. Attempts to establish a homozygous depressor line have not so far met with any success. Four low grade yellows from 14,859 selfed were selfed; all threw some full yellows, giving in all 40 full yellows : 51 depressed; a further six plants from one of these selfings were also tested, and again all gave some full yellows, and a ratio of 15 : 29 in total. In each case the proportions were much the same as from the original depressed yellows, 14,859 and 14,862, which were known to be heterozygotes in view of their backcross origin. All the above selfings total to 96 full yellows : 140 depressed. The deficiency of depressed segregates is even too great to explain on any hypothesis of inviability of the homozygote ( $\chi^2_{2,1} = 5.7$ ). On a normal single gene interpretation the probability of having found at least one homozygote amongst the 10 depressed yellows tested is as high as 0.98. A further five plants will be tested next season; these have been examined by Dr J. Pisk of this laboratory, who has found no abnormality in their male meiosis which might account for the genetic situation. The backcrosses, and those reported below, which also showed normal behaviour, were all from the heterozygote as female, so there is no information on the gametic ratio on the male side. This is under investigation, as additional data are very desirable. There was, within each of the two main classes, a little more variation in selfings than there was in backcrosses, and possibly some of the apparent deficiency may be attributed to errors in scoring, but it is not believed that this can explain the situation, as on the whole there was a perfectly clear distinction between the two groups. As this lowering of yellow intensity was found

to exert a very profound influence on the expression of anthocyanin in the petal, as will appear later, it is proposed, in order to facilitate the discussion, to allocate the symbol  $Ydp$  to the gene responsible, though in view of the selfing results it is realized that the situation may not be as simple as the backcross data suggest.

Two of the depressed yellow segregates from the cross  $14,859 \times N\ 14$  mentioned above were backcrossed to  $N\ 14$  white, and gave three clear classes in very good independent 1 : 1 ratios for both  $y_a$  and  $Ydp$ :

Family	Full yellow	Depressed yellow	White	Total
3408	41	36	78	155
3409	36	47	73	156
Total	77	83	151	311

Evidently  $Ydp$  is completely independent of the  $Y_a$  locus. It could not of course be situated at the  $Y_c$  locus in *anomalum*. That  $Ydp$  is also independent of the Chinese pale locus  $Y_b$  was shown by the segregation in two families derived from depressed yellow plants from the cross  $14,859 \times N\ 44$  ( $Y_b Y_b$ ,  $Ydp ydp \times Y_b^P Y_b^P$ ,  $ydp ydp$ ). These plants were backcrossed to the double recessive  $N\ 44$ ; full yellows and depressed yellows appeared in equal numbers, and the influence of the depressor gene could also be seen within the Chinese pale segregates, approximately half of which were distinctly paler than the others:

Family	Full yellow	Depressed yellow	Chinese pale	Depressed pale	Total
3708	40	34	47	43	164
3709	16	22	12	14	64
Total	56	56	59	57	228

Very few backcrosses to *anomalum* have been grown, and as far as corolla colour is concerned only one large progeny. This, the result of selfing a plant from the second backcross of  $H\ 10$  to *anomalum*, and carrying the  $Y_c$  allele from the former type, segregated 53 plants of grades 6-5-4 and 27 of *anomalum* grade 2. The yellows went rather higher than might have been expected on a presumably homozygous  $Ydp$  background, though there was no evidence that  $ydp$  from  $H\ 10$  had not also been carried over into this line. Scoring of exact yellow grade was very difficult on account of the wide range in intensity of pink in the petal. The point of greatest interest in this progeny was that it showed an excess of pales—53 : 27—which though not significant suggests a tendency for a foreign gene to be in defect when introduced into *anomalum* also.

The depressor gene is of sufficient potency to account for practically all of the downward extension of the great range in corolla colour segre-

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gation which characterizes *anomalum* hybrids as against those between the two cultivated Asiatic species. As far as modifiers affecting the upper portion of the range in yellow intensity are concerned, *anomalum* is probably not very different from many of the *herbaceum* types which are at a lower level than *arboreum* in this respect.

### (5) *The effect of $Y_c^P$ and $Y_{dp}$ on petal length*

In view of the influence of the  $Y_a$  series of alleles on flower size, petal length was measured in certain segregating families, and the results are summarized in Table 6. The first three families all agreed in showing that

Table 6. *The effect of  $Y_c^P$  and  $Y_{dp}$  on petal length*

Family	Corolla colour	No. of plants	Mean petal length mm.	s.e.	Diff.	t	P
3rd B.C. A 8 selfed	Yellow	54	33.14	—	3.37	2.0	0.05-0.02
	<i>anomalum</i> pale	9	29.77	—			
(A 16 × 9992) × 9992	Yellow	206	35.59	—	5.57	16.0	Very small
	<i>anomalum</i> pale	143	30.02	—			
(N 44 × 9992) selfed	Full yellow	63	43.55	0.52	10.89	8.6	Very small
	<i>anomalum</i> pale	9	32.66	1.15			
	Chinese pale	17	44.47	0.80			
	Double recessive	6	38.66	1.23			
(N 44 × 14,859) × N 44	Full yellow	55	45.78	0.39	0.73	1.4	0.2 -0.1
	Depressed yellow	55	45.05	0.33			
	Chinese pale	57	44.98	0.36			
	Chinese pale depressed	57	43.82	0.39			
14,859 × N 14	Full yellow	56	40.17	—	1.47	3.2	Small
	Depressed yellow	60	38.70	—			

the *anomalum* pale segregates have petals from 10 to 25% shorter than those of their full yellow sibs. In each case the difference was significant, and its magnitude was comparable with that which Hutchinson (1931) found to be associated with white corolla in the series which he studied. From the third and fourth families it will be seen that Chinese pales are more like the common pale which Hutchinson studied, in not being very different in petal length from yellows. In the third family the Chinese pales were 0.92 mm. longer than the yellows, but the standard error of this difference was 0.95. In the fourth family they were 0.80 mm. shorter, with a standard error of 0.54. In each case the differences were non-significant. In the third family the double recessives were found to be intermediate in petal length between *anomalum* pale and Chinese pale. They were 6.00 mm. longer than the former ( $t=3.5$ ,  $P$  very small), and 5.81 mm. shorter than the latter ( $t=4.0$ ,  $P$  very small), but in view of their small number it is doubtful whether much significance can be attached to their measurements.

In the fourth family the depressor class was shorter than its corresponding full yellow or Chinese pale class in each case, though only in the latter comparison was the difference significant. Comparison of the petal length of the depressed Chinese pales with that of the full yellows showed a more marked difference, the former being 1.96 mm. shorter than the latter ( $t=3.5$ ,  $P$  very small). This can no doubt be attributed to the cumulative effects of the Chinese pale and the depressor, which separately are small and not easily demonstrable. In the fifth family the depressed yellows again had slightly shorter petals than the full yellows, and in this case the difference was clearly significant. Evidently  $Ydp$  is similar in this respect to the petal colour modifiers which Hutchinson studied.

(6) *Linkage tests against  $Y_c$  and  $Ydp$*

Since markers for these loci have not previously been available in Asiatic cottons, correlations with other gene segregations have been observed whenever possible, and are recorded in Tables 7 and 8. In none of the cases examined was there any evidence of linkage with either  $Y_c$  or  $Ydp$ .

Table 7. *Two-factor selfed segregations involving  $Y_c$*

Factor	Family	Parental constitution	$Y_cX$	$Y_cx$	$Y_c^P X$	$Y_c^P x$	$T$	$\chi^2_L$
$L$	an. 3rd B.C. A 8, S	++ × --	55	13	10	2	80	0.1
	14,868 × N 5, S	+ - × - +	92	30	18	11	151	2.1
	9992 × N 44, S	+ - × - +	62	21	12	3	98	0.2
$Lc_1$	14,868 × N 5, S	+ - × - +	90	19	21	4	134	0.1
	9992 × N 44, S	+ - × - +	57	15	7	6	85	3.8
$R_2$	14,868 × N 5, S	+ - × - +	91	31	25	6	153	0.5
$H_a$	9992 × T 6, S	+ - × - +	44	16	11	5	76	0.1

Table 8. *Two-factor backcross segregations involving  $Ydp$*   
( $F_1$  used as female)

Factor	Family	Parental constitution	$YdpX$	$Ydp x$	$ydpX$	$ydp x$	$T$	$\chi^2_L$
$L$	(14,859 × N 14) × N 14	++ × --	39	43	36	41	159	0.1
	(14,859 × N 44) × N 44	++ × --	56	56	54	61	227	0.2
$Lc_1$	(14,859 × N 14) × N 14	++ × --	36	42	41	34	153	1.1
	(14,859 × N 44) × N 44	++ × --	56	51	55	47	209	0.1
$R_2$	(14,859 × N 14) × N 14	++ × --	42	41	32	45	160	1.3
$Ne$	(14,859 × N 14) × N 14	++ × --	45	37	31	46	159	3.4

(7) *Summary*

(i) With respect to flower colour, *G. anomalum* is complementary with *arboresum* strains carrying the common pale or white allele in the  $Y_a$  locus, or Chinese pale in the  $Y_b$  locus. To the gene responsible for the pale corolla of *anomalum* the symbol  $Y_c^P$  is assigned. All strains of *arboresum*

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and *herbaceum* carry its allele  $Y_c$ . The constitution of *anomalum* is  $Y_a Y_b Y_c^P$ .

(ii) Although the  $Y_c^P$  pale corolla, when in *arboreum*, is not very different in appearance from the  $Y_a^P$  pale, it is more like  $y_a$  white in being associated with a considerably smaller flower than the full yellow types.

(iii) All *anomalum* hybrids, even those involving full yellow cultivated Asiatic parents, are alike in the low intensity of yellow in the corolla. This is due primarily to a yellow depressor from *anomalum*, to which the symbol  $Ydp$  is allocated. This gene lowers the intensity of full yellow in *arboreum* from grade 7-8 to grade 4.  $Y_c^P$  pale is of grade 3 in an *arboreum* genotype ( $ydp$ ), but in *anomalum* ( $Ydp$ ) the expression of this gene is reduced to grade 2 by the depressor.

(iv) The depressor, like other corolla colour modifiers, restricts petal length to a very slight extent.

(v) *Anomalum* hybrid progenies show a very much wider corolla colour modifier segregation than do crosses between the two cultivated Asiatic species. Most of the downward extension in range of yellow intensity is due to the  $Ydp$  gene.

(vi) The  $Y_c$  locus is independent of  $Y_a$ ,  $Y_b$ ,  $R_2$ ,  $H_a$ , and the  $L-Lc_1$  linkage group.  $Ydp$  is independent of  $Y_a$ ,  $Y_b$ ,  $Y_c$ ,  $R_2$ ,  $Ne$ , and the  $L-Lc_1$  group.

### IV. ANTHOCYANIN

#### (1) *The situation in the cultivated Asiatic species; symbolization*

In *G. arboreum* and *herbaceum* anthocyanin distribution is controlled by a lengthy series of multiple allelomorphs. Six types were originally described by Hutchinson (1932*b*) and the genes arranged serially:

$$R-R^L-R^C-R^S-r^s-r^o.$$

$R^S$  is by far the commonest allele, and the red spot at the base of the petal and the variable intensities of red tinge on the stem which it produces might be described as the typical condition in cotton (Pl. 18, fig. 1). The  $r^o$  type is completely spotless (Pl. 18, fig. 2); the red tinge on the stem of the strain in which this gene occurs is slightly paler than that on most  $R^S$  types, but this is almost certainly due to modifiers and not to any difference in the main gene in this respect. The  $r^s$  type, ghost spot (Pl. 18, fig. 3), has a clear white area at the base of the petal in the position which is occupied by the red spot in  $R^S$  types. Ghost spot types however not only lack anthocyanin from the petal spot, but from the entire vegetative part of the plant as well. The two alleles  $r^o$  and  $r^s$  act as complementaries in that their compound (Pl. 18, fig. 14) has a similar

expression to that of the next higher member,  $R^S$ . Hutchinson described this compound as "spot/ghost" on account of the pale whitish margin surrounding the pigmented area, as if the red spot were superposed on a slightly larger ghost. Evidence which will be presented in this paper shows that this expression is not an essential characteristic of the compound, which on certain genetic backgrounds is intensified until it is quite indistinguishable from  $R^S$  phenotypically. The higher members of the series,  $R^C$ ,  $R^L$ , and  $R$ , are also, like  $R^S$ , characterized by a red petal spot, but in addition determine progressive extension of intense anthocyanin pigmentation first to the calyx and bolls, next to the leaves as well, and finally also to the petal lobe. Subsequently Hutchinson & Ghose (1937*b*) reported a seventh member of the allelic series,  $R^o$ , similar to  $R^L$  vegetatively, but, like  $r^o$ , without any petal spot, and in this respect also complementary with  $r^S$ . This, together with information obtained in the course of this investigation with *anomalum*, has led to the realization that the anthocyanin alleles may be arranged in two series, similar in vegetative expression but respectively with and without red petal spot. On this basis a new system of nomenclature of the genes has been proposed (Hutchinson & Silow, 1939). Two superscripts are attached to the main  $R$  symbol; the first indicates distribution of anthocyanin if present, and the second, presence or absence of spot. In the following list the new symbols are indicated, together with the old ones in brackets. As Harland (1935) has established homology of this series with the  $R_2$  series in the tetraploid New World cottons, this series is also given the same numerical subscript.

	Petal spotted	Petal spotless
Red plant body and petal	$R_2^{RS}$ ( $R$ )	
Red leaf	$R_2^{LS}$ ( $R^L$ )	$R_2^{LO}$ ( $R_2^o$ )
Red calyx	$R_2^{CS}$ ( $R^C$ )	
Red tinged stem (i.e. basic anthocyanin present, expression variable but slight)	$R_2^{AS}$ ( $R^S$ )	$R_2^{AO}$ ( $r^o$ )
Green stem, ghost spot (basic anthocyanin absent)	$R_2^{OS}$ ( $r^S$ )	

This scheme of symbolization indicates the basis of the complementary nature of the ghost spot ( $R_2^{OS}$ ) and spotless ( $R_2^{AO}$  or  $R_2^{LO}$ ) in giving the red spot phenotype. In the course of discussion in this paper it is proposed to refer to the phenotype of  $R_2^{AS}$  as "full spot", and to the compound between ghost  $R_2^{OS}$  and spotless  $R_2^{AO}$  as "compound spot". The latter term will also be used for compounds between ghost and spotless when such genes occur in different loci, as will be shown to be the case in *anomalum*. In cases where there is no immediate evidence as to whether a particular phenotypic grouping is composed of full spot, or compound,



or both, or where the distinction is immaterial to the point under discussion, the class will be referred to as "red spot".

From Hutchinson's earlier work it had been thought likely that the compound spot would usually be distinct in expression from the full spot allele  $R_2^{AS}$  in having a clear white marginal area around the spot (see Pl. 18, fig. 14), though he had reported the appearance of a single intensified spot/ghost plant which was hardly distinguishable from a typical full spot, and suggested that it might be possible to develop a spotless type with a complete set of *arboreum* modifiers which in compound with ghost would give a full spot phenotype. A large number of progenies which have been grown from *anomalum* interspecific hybrids have included both the ghost and spotless alleles, so considerable attention was focused on the scoring of the compound as distinct from the full spot, especially in early progenies when *anomalum* was thought to carry a full spot duplicate. The separation was found to be exceedingly difficult, and expected ratios were not attained. Spot/ghost types appeared even in progenies where only *anomalum* and Asiatic full spots had entered. These difficulties of interpretation were eventually explained by the demonstration that the *anomalum* spot is itself due to the complementary reaction between ghost and spotless, though these two genes are in different loci, and that compounds can be intensified by modifiers right up to the expression of full spot. In such cases it is now evident that there would be no point in maintaining the distinction between compound and full spot, and the data have been grouped as "red spot".

It will be shown that *anomalum* carries a duplicate anthocyanin locus. On account of sterility barriers it has not been possible to investigate the relationship of this duplicate locus with the  $R_1$  series of New World cottons. From work in progress at this Station it has been established that the anthocyanin locus of the wild American diploid species *G. armourianum*, *aridum*, and *thurberi* is homologous with  $R_1$  of the tetraploid American species. Skovsted (1934) has suggested that the latter are allopolyploids derived from an Asiatic species and an American wild species, or cytologically similar types. On this basis  $R_1$  would not be expected to occur in an Asiatic genom, and it is therefore proposed tentatively to symbolize the *anomalum* anthocyanin duplicate locus as  $R_3$ .

Since it has been possible to transfer the  $R_3$  gene from *anomalum* to the cultivated Asiatics, and obtain normal segregation, it is highly probable that the latter species also carry the  $R_3$  locus. There is of course a possibility that the *arboreum-herbaceum* homologue of the *anomalum*  $R_3$  chromosome has a deletion at this locus, but in the absence

of any positive evidence to this effect, it is more rational to suppose that the locus is present. Within the cultivated Asiatic species there is however no evidence of its activity. The  $R_2^{OS}$  type does not carry  $R_2^{AO}$ , nor does the  $R_2^{AO}$  type carry  $R_2^{OS}$ , since, on evidence which will be brought forward in this paper, these combinations of genes would also be complementary in duplicate loci just as they are when in the same allelomorphic series. There is no evidence that these spotless and ghost types carry the same alleles in each of their duplicate loci, since in the  $F_2$  between them they would give 14 spot : 1 ghost : 1 spotless, whereas they actually give 2 : 1 : 1. The duplicate locus in *arboresum* and *herbaceum* must therefore carry an allele which is inactive so far as anthocyanin expression is concerned— $r_2^{oo}$ —a new spotless allele phenotypically similar to  $R_2^{AO}$  but lacking even the basal anthocyanin episome and therefore not complementary with  $R_2^{OS}$ .

When it became known that *anomalum* carries a duplicate spot mechanism, careful watch was kept for any evidence of duplication for anthocyanin in the cultivated Asiatic species, especially in the African types *G. arboresum* forma *soudanensis* and *G. herbaceum* var. *frutescens* and var. *africanum*. None has been found.

## (2) Evidence of duplication

With reference to anthocyanin *G. anomalum* (Pl. 18, fig. 4) is phenotypically similar to full spot  $R_2^{AS}$  types in the cultivated Asiatic species (Pl. 18, fig. 1), with four of which it has been crossed—A 8 and N 19 from *arboresum*, and O 1 and O 8 from *herbaceum*. The hybrids were backcrossed to several *arboresum* ghost types, and the progeny segregated for petal spot as follows:

Cross	Red spot	Ghost	Total	$\chi^2_{3:1}$	P
( <i>an.</i> × A 8) × A 16	8	1	9	—	—
( <i>an.</i> × A 8) × N 14	174	28	202	13.4	Very small
( <i>an.</i> × A 8) × T 3	103	35	138	0.0	0.95–0.90
( <i>an.</i> × A 8) × T 14	6	0	6	—	—
( <i>an.</i> × N 19) × T 1	37	22	59	4.8	0.05–0.02
( <i>an.</i> × O 1) × N 14	8	3	11	—	—
( <i>an.</i> × O 8) × N 5	8	1	9	—	—
( <i>an.</i> × O 8) × N 14	15	7	22	—	—

The occurrence of recessive ghosts in a backcross involving two dominant phenotypes clearly indicated duplication. There was considerable heterogeneity amongst the families ( $\chi^2$  heterogeneity = 19.1,  $P = 0.01$ ). Of the three larger families, one was an excellent fit to the 3 : 1 expectation, whilst the other two gave a very significant excess and deficiency of ghosts respectively. In such a wide cross and with so much sterility this was not altogether unexpected. The duplicate gene interpretation

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was substantiated by subsequent breeding behaviour of spotted selections from the backcross (*an.*  $\times$  A 8) to N 14, which were again backcrossed to N 14 ghost.

Plant	Red spot	Ghost	Ratio	$\chi^2_{3:1}$	$\chi^2_{1:1}$
P 1780	285	108	2.64 : 1	1.3	—
P 1781	138	43	3.21 : 1	0.1	—
P 1774	61	58	1.05 : 1	—	0.1
P 1783	94	93	1.01 : 1	—	0.0
P 1795	111	90	1.23 : 1	—	2.2
P 1797	47	52	0.90 : 1	—	0.3

One in three of these would have been expected to be double heterozygotes, and the other two single heterozygotes giving 1 : 1. A seventh plant, P 1772, was selfed and gave 51 red spot : 5 ghost, indicating that it also was a double heterozygote.

Some selections from the third backcross of (*an.*  $\times$  A 8) to A 8 were also of interest in this connexion. A 8 being homozygous full spot, the backcross progeny were all phenotypically spot. Two selections when selfed gave the following:

Plant no.	Red spot	Ghost
14,854	54	9
14,864	55	3

These two plants evidently were heterozygous in both the A 8 full spot locus and in the duplicate locus, giving approximations to the 15 : 1 ratio. One red spotted segregate, no. 9992, from the first of these selfed progenies, also turned out to be a double heterozygote like its immediate parent. It was crossed with A 16 ghost, and the five progeny which were grown were all red spotted. On the basis of duplicate factors, one-third of these spotted progeny should have been double heterozygotes, and two-thirds single heterozygotes. They were crossed, as mentioned in § III in connexion with corolla colour analysis, back to their 9992 parent, the double heterozygote, with which the double heterozygotes should have given a 15 : 1 ratio, and the single heterozygotes a 7 : 1. Two of the families appeared to be of the former type, and three of the latter:

Cross	Red spot	Ghost spot	Ratio	$\chi^2_{15:1}$	$\chi^2_{7:1}$
P 4098 $\times$ 9992	50	4	12.5 : 1	0.1	1.3
P 4126 $\times$ 9992	78	4	19.5 : 1	0.3	4.4
P 4127 $\times$ 9992	64	8	8.0 : 1	2.9	0.1
P 4128 $\times$ 9992	68	12	5.7 : 1	10.5	0.5
P 4129 $\times$ 9992	66	7	9.4 : 1	1.4	0.6

Plant 9992 was also crossed with N 5 ghost, and one of its spotted progeny gave further evidence of duplication. On selfing it gave 49 red spotted and 3 ghost spot progeny (15 : 1) and a backcross to ghost gave 52 red spot and 12 ghost (3 : 1). Abundant other evidence of duplication for spot will appear incidentally later in this paper.

(3) *The  $R_2$  allele in anomalum*

When it became known that the *anomalum* spot is a duplicate of that in *arboreum* and *herbaceum*, it was necessary to determine the nature of the allele which *anomalum* carries in the *arboreum* anthocyanin locus ( $R_2$ ). The fact that ghost segregates appeared in the backcrosses of (*an.*  $\times$  Asiatic full spot) to ghost  $R_2^{OS}$  types indicated that the  $R_2$  allele from *anomalum* must be either ghost or a lower member. Accordingly four of the ghost segregates, nos. P 1791, P 1801, P 1818, and 11,319, from the above (*an.*  $\times$  A 8)  $\times$  N 14 backcross, were crossed to H 10 spotless  $R_2^{AO}$ . They gave a total of 316 plants all with the red spot phenotype characteristic of the ghost-spotless compound, indicating that their ghost parent must have been homozygous for the ghost allele. That the *anomalum*  $R_2$  contribution is a ghost allele was also confirmed by the following line of evidence. The hybrid (*an.*  $\times$  A 8) was itself backcrossed to H 10 spotless  $R_2^{AO}$ , and all 550 progeny grown were red spotted. This should be contrasted with the 3 red spot : 1 ghost segregation which occurred on backcrossing the same hybrid to ghost types. Two plants, nos. 11,703 and 11,757, were selected from the H 10 backcross for further testing. On again backcrossing they gave 1 : 1 and not 3 : 1 ratios, indicating that the duplicate spot from *anomalum* was not present in these selections. Attention need therefore be directed to the  $R_2$  locus only, at which, in addition to the spotless allele derived from H 10, these selections might have carried full spot from A 8, in which case a backcross to ghost would have given all spotted progeny; actually about 50 % ghost were obtained, so their second  $R_2$  member must have been ghost, as was confirmed by backcrossing these same two selections again to spotless. This ghost could only have been derived from *anomalum*. The actual numbers obtained in these backcrosses of 11,703 and 11,757 ( $R_2^{AO}$   $R_2^{OS}$ ) were as follows:

Cross	Segregation		$\chi^2_{1:1}$	P
11,703 backcrossed ghost ( $R_2^{OS}$ )	220 compound spot ( $R_2^{OS}$ $R_2^{AO}$ )	149 ghost ( $R_2^{OS}$ $R_2^{OS}$ )	13.7	Very small
11,757 ditto	25 ditto	21 ditto	0.3	0.7-0.5
11,703 backcrossed spotless ( $R_2^{AO}$ )	52 spotless ( $R_2^{AO}$ $R_2^{AO}$ )	35 compound ( $R_2^{AO}$ $R_2^{OS}$ )	3.3	0.1-0.05
11,757 ditto	172 ditto	139 ditto	3.5	0.1-0.05
Total	469 $R_2^{AO}$	344 $R_2^{OS}$	20.8	Very small
Heterogeneity			1.6	0.7

In all four progenies plants carrying the introduced ghost gene  $R_2^{SO}$  of *anomalum* origin were fewer in number than expected. The families formed a homogeneous group, and although in only one of them was the deficiency actually significant, in total there was a very significant

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shortage of plants carrying the introduced gene—a frequent tendency to which attention will be directed again later.

The plants 14,854 and 14,864, to which reference has already been made in the preceding section, also showed that the  $R_2$  allele in *anomalum* is ghost. They were selections from a third backcross of (*an.*  $\times$  A 8) to A 8, and threw ghosts in their selfed progeny. Since A 8 is homozygous  $R_2^{AS}$ , the  $R_2^{OS}$  gene must have come from *anomalum*.

### (4) *The complementary nature of the duplicate in $R_3$ , and its pleiotropic "gold petal" effect*

The knowledge that *anomalum* carries ghost in the  $R_2$  locus raised the possibility that the duplicate might not necessarily be a full spot allele on its own account, but a spotless gene giving the spot phenotype in association with ghost. Critical evidence is available from the backcross of the hybrid (*an.*  $\times$  H 10 spotless) to H 10. If the duplicate were a full spot, it should have given a 3 : 1 duplicate gene ratio, since the *anomalum*  $R_2^{OS}$  would also have acted as a spot phenotype on the H 10 spotless background. Actually 29 red-spotted and 28 spotless plants were obtained, clearly a 1 : 1 ratio, which can only be interpreted on the basis that the *anomalum* spot is due to complementary alleles, respectively ghost and spotless, in duplicate loci, as follows:

Parents	<i>anomalum</i> spot	$R_2^{OS} R_3^{AO}$	H 10 spotless	$R_2^{AO} r_3^{OO}$
$F_1$		spot	$R_2^{OS}$	$R_3^{AO} r_3^{OO}$
$F_1$ gametes	$R_2^{OS} R_3^{AO}$	$R_2^{OS} r_3^{OO}$	$R_2^{AO} R_3^{AO}$	$R_2^{AO} r_3^{OO}$
Phenotypes in B.C. to H 10 $R_2^{AO} r_3^{OO}$	Spot	Spot	Spotless	Spotless

It was not practicable to grow a larger backcross family, since many *herbaceum* types, including H 10 which was the only spotless available at the time of these experiments, are not well adapted to the local climate. The difficulty of obtaining a good stand was increased by the high sterility of the hybrid. There is however further very conclusive evidence of the spotless nature of the *anomalum*  $R_3$  allele, and this was derived from the hybrid between *anomalum* and the *arboreum* ghost type A 16. Since *anomalum* also carries ghost in the  $R_2$  locus, this hybrid was homozygous for  $R_2^{OS}$ , as was confirmed by a backcross to H 10 spotless, in which all 241 progeny were spotted. On this account all spotted progeny in recurrent backcrosses to A 16 were of the same main genotype for spot as the original (*an.*  $\times$  A 16) hybrid. This point is of considerable importance in facilitating subsequent analysis.

Although *anomalum* itself (Pl. 18, fig. 4) has only the faintest suggestion of pink on the petal lamina, all of its hybrids were characterized by a distinct tinge of pink overlying the otherwise yellow corolla (Pl. 18,

figs. 5-7), giving a gold or bronze appearance to the petal which had never before been seen in Asiatic cottons. The degree of expression of gold was very different in the several hybrids which have been grown, and was strongest in the A 16 cross (Pl. 18, fig. 7). Practically all red-spotted segregates in each of the successive backcrosses to A 16 were also characterized by gold appearance to some extent, whereas all ghost segregates had a perfectly clear yellow petal as in A 16 itself (Pl. 18, fig. 3). This association of spot with gold petal has been carefully followed through wherever possible. In the early days of this investigation, before the significance of gold petal expression was fully appreciated, a first backcross of (*an.*  $\times$  A 16) to A 16 was classified as 20 red spot : 30 ghost. All of the latter were absolutely clear yellow petal, and in progeny derived from these and other ghost segregates gold petal has never appeared. Fifteen of the red-spotted plants were noted as having the gold expression in varying degrees from intense to very faint, and only one was scored as clear yellow petal. For the other four there was no definite record of gold, attention having been distracted in the case of two of them to the expression of the yellow depressor. The remaining two had very small abnormal petals on which it was difficult to determine colour exactly, but gold occurred in the selfed progeny of one of them. Three of the red-spotted gold-petalled plants of this first backcross were again backcrossed to A 16, giving 11 red spot : 6 ghost. All of the latter were yellow petal, whilst 8 of the red spot were classified as gold. Of the remaining three not recorded for gold, one had small abnormal petals; the other two in subsequent progeny showed gold petal red spot, so again must have been gold petal genotypically if not phenotypically.

Seven of the red-spotted plants of the second backcross when backcrossed again to A 16 (third backcross) gave 49 red-spotted all with gold, and 60 ghost all clear yellow petal. Three red-spotted gold-petal plants of the third backcross, nos. 15,094, 15,095 and 15,099, were selfed, and gave respectively 46 : 23, 22 : 9, and 14 : 8 red-spotted and ghost-spot segregates. All of the latter were clear yellow, and all except one of the red-spotted plants were gold petalled. This exception which occurred in the progeny of plant no. 15,094 was of the same type as the remainder of the family, but could not be rechecked when its aberrant scoring was noted. However 15,094 was again selfed and backcrossed, giving in the respective families 29 : 4 and 20 : 28 red-spotted and ghost-spot plants. All of the spotted class showed gold petal. It is therefore very likely that the exception noted in the earlier sowing was incorrectly graded. The gold was still variable in intensity in the third and fourth backcrosses,

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though present in some degree in every one of the spotted segregates. It may reasonably be concluded that gold petal is constantly associated with the *anomalum* red-spot class, and it has never been observed in ghost segregates.

In this connexion the following evidence is of particular significance. At the time when the above constant association was first suspected, a red-spotted selection, no. 14,544, from the third backcross of (*an.* × H 10) to N 14 was available. N 14 has both white petal and ghost spot; 14,544 also had a white corolla, and its breeding behaviour had shown that it was also homozygous for ghost in the  $R_2$  locus, and heterozygous in the  $R_3$  locus for the spotless allele derived from *anomalum*, to whose interaction with ghost its red-spot phenotype was due. Yet no gold was visible on the petal, as a result of the absence of yellow flavone. It was therefore of interest to determine if gold were really latent in this plant, so it was crossed with A 16 ghost, homozygous for yellow corolla. The progeny consisted of 85 with ghost spot and absolutely clear yellow petals, and 83 with red spot and a distinct though faint tinge of gold. The low intensity of gold was later found to be a result of heterozygosity for white corolla; the important point is that gold was present on all plants which showed the *anomalum* spot, and in that respect this evidence strongly supports the conclusions drawn from the A 16 backcross lines.

Reverting to the latter, a gold-petalled red-spotted plant was selected from the second backcross of (*an.* × A 16) to A 16. This plant must have been homozygous for ghost, since both *anomalum* and A 16 carry this gene in the  $R_2$  locus, and heterozygous for the *anomalum*  $R_3$  spotless allele to which its red-spot phenotype was due. This was crossed with H 10 spotless, and a small progeny grown. As a result of the interaction with ghost, all progeny were spotted, and two with gold petals, P 2381 and P 2386, were selected. These were backcrossed to H 10, when it was found that not only were some of the red-spotted progeny gold-petalled, but some of the spotless segregates also showed gold of varying intensities. The scoring was as follows:

Cross	Red spot		Spotless		$\chi^2_{1:1}$	
	Gold	No gold	Gold	No gold	Spot	Gold
P 2381 × H 10	40	23	39	32	0.5	4.3
P 2386 × H 10	116	140	96	126	2.4	6.1

The families were somewhat dissimilar in their segregation ( $\chi^2$  heterogeneity = 11.14,  $P = 0.01$ ); in the first family the spot segregation was near the 1 : 1 expected (63 : 71), but in the second family there was a fairly large excess of spotted plants (256 : 222). In the first family there was

a significant excess of golds (79:55) and in the second a significant deficiency (112:266), and to this the heterogeneity between the families was due. No explanation of the aberrant ratios can be brought forward. It can only be mentioned that other obviously monogenic segregations were also frequently distorted in this hybrid material, and in no case has breeding behaviour been found to be confictory with the genic interpretation suggested. The point of importance is that both of the above families agreed in showing complete independence of spot and gold ( $\chi^2_L$  for total deviation 0.4,  $P=0.5$ , for heterogeneity 0.4,  $P=0.5$ ). This independence was in striking contrast to the segregations previously discussed, and nothing like the gold spotless type, which is shown in Pl. 18, fig. 13, had ever previously been seen in the Asiatic cottons. The basal area of the petal, corresponding to the portion occupied by red spot or ghost spot in other cottons, was clear yellow, but the remainder of the petal lamina was of the same gold tinge as had previously been associated with the *anomalum* spotted types in the A 16 backcross lines. The occurrence of gold spotless types, the complete independence of gold from the  $R_2$  spot segregation in this H 10 backcross, and its absolute association with the *anomalum*  $R_3$  spot phenotype in the A 16 backcross lines, can only be explained on the basis that the *anomalum*  $R_3$  allele is itself spotless, gives the complementary spot reaction with ghost, and is responsible for the development of gold petal. This conception is not in any way different from that of the various vegetative red effects which have been shown to be associated with both spot and spotless members of the  $R_2$  series in the cultivated Asiatics. The symbol  $R_3^{GO}$  is proposed for this allele— $G$  in the superscript signifying the presence of basic anthocyanin with gold petal as its special expression; and  $O$  signifying that no spot is present. The above segregations would therefore be interpreted as follows:

Parents	<i>anomalum</i> spot $R_2^{OS} R_3^{GO}$		A 16 ghost $R_2^{OS} r_3^{OO}$	
$F_1$ genotype	$R_2^{OS} R_3^{GO}$		$R_2^{OS} r_3^{OO}$	
Gametes of $F_1$ (and of spotted selections from recurrent B.C.'s to A 16)	$R_2^{OS}$	$R_3^{GO}$	$R_2^{OS}$	$r_3^{OO}$
Phenotypes in 1st B.C. to A 16, $R_2^{OS} r_3^{OO}$ (and in recurrent B.C.'s of spotted selections)	Spot, gold petal		Ghost, no gold	
Phenotypes in progeny of spotted, gold petal selections (same genotype as $F_1$ ) crossed with H 10, $R_2^{AO} r_3^{OO}$	Spot, gold petal		Spot, no gold	
Gametes of spotted, gold petal selections P 2381 and P 2386, $R_2^{AO} R_2^{OS} R_3^{GO} r_3^{OO}$	$R_2^{AO}$	$R_3^{GO}$	$R_2^{AO} r_3^{OO}$	$R_2^{OS} R_3^{GO}$
Phenotypes in B.C. of P 2381 and P 2386 to H 10, $R_2^{AO} r_3^{OO}$	$R_2^{OS} R_3^{GO}$	$R_2^{OS} r_3^{OO}$	$R_2^{AO} R_3^{GO}$	$R_2^{AO} r_3^{OO}$
Phenotypes in B.C. of P 2381 and P 2386 to H 10, $R_2^{AO} r_3^{OO}$	Spotless, gold	Spotless, no gold	Spot, gold	Spot, no gold



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P 2381 and P 2386 were also selfed. On the above basis expectation would be:

Red spot, gold petal	Red spot, no gold	Ghost spot, no gold	Spotless, gold petal	Spotless, no gold
9	2	1	3	1

Actual figures were

	Red spot			Ghost spot	Spotless			Total
	Gold petal	No gold	Gold not scored	No gold	Gold petal	No gold	Gold not scored	
P 2381 selfed	74	29	(3)	3	43	15	(2)	169
<i>Expected</i>	<i>95.1</i>	<i>21.1</i>		<i>10.6</i>	<i>31.7</i>	<i>10.6</i>		<i>169.1</i>
P 2386 selfed	210	43	(4)	20	64	22	(4)	367
<i>Expected</i>	<i>206.4</i>	<i>45.9</i>		<i>22.9</i>	<i>68.8</i>	<i>22.9</i>		<i>366.9</i>

In the first family the fit was not very good.  $\chi^2$  for the three main classes—red spot, ghost and spotless, was high, 13.8, and most of this was due to a deficiency of ghost and an excess of spotless. Within the spotless class the ratio gold : no gold was fairly good, but was somewhat out within the red-spotted class. Expectation was however very closely realized in the second family.

From this material  $R_3^{GO}$  has been established in the homozygous condition in association with  $R_2^{40}$ , giving gold spotless. Three of the deepest gold types, one from P 2381 selfed, and two from P 2386 selfed, all scored as intense, were tested for homozygosity. Two of these selections when selfed gave 49 and 43 plants respectively, all of which were spotless, with medium gold to intense gold or intense pink petal. The third plant, no. 6144, although somewhat more intensely gold than its homozygous sib, turned out to be heterozygous for  $R_3^{GO}$ . It will be remembered that P 2381 and P 2386 were the result of two backcrosses to A 16 followed by one backcross to H 10. The latter strain will be shown later to be low in content of gold intensifying modifiers relative to A 16, so that wide segregation for the modifiers would be expected, and this would to some extent mask the distinction in gold intensity between  $R_3^{GO}$  homozygotes and heterozygotes.

$R_3^{GO}$  has also been established in the homozygous phase as a gold-petalled red-spotted type in association with  $R_2^{OS}$  ghost, i.e. of the same main anthocyanin gene constitution as *anomalum* itself, but on a yellow-petalled *arboreum* genotype. The same seven spotted segregates from the second backcross to A 16 ghost, which as reported above were selected for further backcrossing, were also selfed. In all they gave 37 red-spotted and 20 ghost segregates. All of the red-spotted plants had gold petal, ranging from intense to very intense. Six of the latter type were selfed. Four of them were heterozygotes, giving in all 42 red spot, gold : 21

ghost spot, no gold. Two others were homozygotes. One of these, 15,240 (Pl. 18, fig. 9), gave a small selfed progeny of eleven plants, all red-spotted, intense to very intense gold petal. Its homozygosity was further confirmed by backcrosses to ghost, which gave 85 progeny, all red-spotted. The other homozygote, 15,245, did not give any selfed seed, but in crosses with ghost gave 145 progeny, all red spotted. These red-spotted  $R_2^{OS} R_3^{GO}$  homozygotes were more intensely gold than the spotless  $R_2^{AO} R_3^{GO}$  ones, since the latter carried part of the low-grade gold genotype of the H 10 strain.

(5) *Variability in gold petal expression*

In presenting the evidence for the constitution of *anomalum* with reference to corolla colour and anthocyanin each particular factor has been discussed separately. Whilst this greatly facilitates analysis, it does not give any idea of the extreme variability within the early segregating progenies, nor of the great differences in range of variability between those derived from different hybrids. Not only was there the wide range in shade of yellow, even in the absence of the main gene segregation, as was shown in Table 4, but superposed on this there was also a wide segregation for gold or pink, together with great variability in size and intensity of the petal spot, ranging from very minute to one which covered about half of the petal surface. Attention will first be directed to the variability in gold expression. Its association with the *anomalum* anthocyanin allele has been illustrated from material derived from the *an.*  $\times$  A 16 hybrid, of which a small first backcross was cited. Later a larger first backcross was grown, and its classification for yellow corolla grade and for pink is shown in Table 9.

Table 9. *Segregation for yellow corolla grade, and for spot and the associated gold or pink expression, in the first backcross (an.  $\times$  A 16) to A 16*

		Yellow corolla grade							Total
		8	7	6	5	4	In-definite*	Not scored	
Red spot	Petal very pink or with deep pink edge	.	1	.	1	1	2	.	5
	Medium pink	.	2	.	4	4	1	.	11
	Faint pink	.	.	.	2	5	.	.	7
	Very int. or int. gold	.	4	.	.	.	12	.	16
	Medium gold	2	11	2	1	.	6	.	22
	Faint gold	.	7	1	.	.	8	.	16
	Very faint gold	.	5	1	.	3	3	.	12
	Gold not recorded	.	.	.	.	.	.	41	41
Ghost spot	(No gold	29	18	1	3	.	.	17	68
	Gold not recorded	.	.	.	.	.	.	37	37

\* Yellow grade obscured by gold or pink.

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This table adequately demonstrates the complexity of segregation of early backcrosses. Scoring of pink or gold on the corolla was difficult. Four rather indefinite grades were used—very intense to intense, medium, faint, and very faint. Very intense gold corresponds to fig. 9 in Pl. 18, medium gold corresponds to fig. 10, and very faint gold to fig. 11. Fig. 12 represents the intense pink grade, though in this family many of the pinks were less uniformly coloured over the petal surface, with a strongly marked tendency to deepening towards the petal edge where the petal had been exposed to the sun in the bud stage. Although the scoring for any particular plant was somewhat variable, it was fairly consistent. The greater part of the variability within a plant appeared to be due to differences in exposure of the bud to the direct sun. It was also difficult to score accurately the wide segregation in corolla grade on account of the variation in the overlying pink or gold. Pink as distinct from gold was not merely the same intensity of anthocyanin overlying a paler grade of yellow, but there was a real intensification of anthocyanin pigment in the petal lobe, as may be seen by comparing figs. 10 and 12 in Pl. 18; the plants from which these figures were painted were sibs of known and similar constitution except for the presence in the pink plant of the *Ydp* gene which has been discussed in the corolla colour section, and which will be shown to be responsible for the distinction between gold and pink.

The fact that in the A 16 backcross lines there were only two main anthocyanin genotypes segregating facilitated recognition of the association between gold and the spotted class, though from Table 9 it will be seen that there was great variability in expression of gold within this class. Since so many of the backcrosses involving other *arboreum* and *herbaceum* types did not manifest gold petal to anything like the same extent as did backcrosses to A 16, doubts arose at one time as to whether gold had really come in from *anomalum*, and to make sure that A 16 was not in some obscure way the source of it, A 16 itself was crossed with 14,859, a plant carrying the gene *Ydp* which had been found to accentuate the expression of gold to pink; the thirteen plants derived from this cross showed no sign of pink, confirming that A 16 was not the source of gold. It was eventually realized that A 16 was at a rather higher modifier level than most other types used in this study, but this situation in itself was not sufficient to explain all the observed variability in expression of gold, and an attempt was therefore made to determine what other factors were involved.

The two red-spotted plants 15,240 and 15,245, from the second back-

cross selfed of (*an.*  $\times$  A 16) to A 16, and which had been found to be homozygous for the gold spotless allele, were used. They themselves were graded as "very intense gold", and the selfed progeny of one of them graded "intense to very intense", mostly the latter. All were on a homozygous yellow corolla background ( $Y_a Y_a$ ) (Pl. 18, fig. 9). Backcrosses of these two plants to A 8 (81 progeny observed) gave an estimate of the expression of heterozygous gold on homozygous yellow corolla. They graded "medium to intense", mostly the former, as also do red-spotted segregates in backcrosses of heterozygous selections in the A 16 backcross lines (Pl. 18, fig. 10). The indication that gold is not completely dominant therefore accounts for some of the variability of gold in selfed lines carrying  $R_3^{GO}$ .

The same two homozygous gold spotless plants were also crossed with N 5 and N 14. N 5 is a ghost spot pale yellow corolla  $Y_a^P$  strain, and N 14 is a ghost spot white corolla  $y_a$  type. The progeny gave an estimate of heterozygous gold on a heterozygous yellow corolla background. Where N 5 was involved (100 progeny observed), and corolla constitution of the progeny was therefore  $Y_a Y_a^P$ , gold was reduced to very faint (Pl. 18, fig. 11), being hardly discernible except against clear yellow petal types for contrast, in which case there was then no doubt as to its presence. Examination of the paintings will show however that it would not always be easy to see this faint expression in a family with a wide segregation for gold and yellow. In progenies involving N 14 (130 plants examined) gold, though discernible, was even fainter. The hybrids ( $15,240 \times$  N 14), ( $15,245 \times$  N 14), ( $15,240 \times$  N 5) and ( $15,245 \times$  N 5) were extensively selfed in connexion with  $R_3$  linkage studies, and observations were also made on gold expression in these  $F_2$  progenies. The hybrids were of  $R_2^{OS} R_3^{OS} R_3^{GO} r_3^{OO}$  constitution, so that all spotted progeny were of necessity compounds between  $R_2^{OS}$  and  $R_3^{GO}$ , but all white corolla segregates ( $y_a y_a$ ) which carried red spot lacked any trace of gold on the petal. As expected, the red-spotted yellow petal segregates showed a range from very intense to very faint gold, since they were either homozygous or heterozygous for both  $Y_a$  and  $R_3^{GO}$ . Some but not all of the spotted pale corolla ( $Y_a^P Y_a^P$ ) segregates, when contrasted against ghost segregates, showed an extremely slight indication of gold. The pales ranged from grades 3-1½ in these families, and there was a tendency for the gold to be somewhat more definite on the higher grade backgrounds. All this evidence showed that the expression of gold is very dependent on corolla colour constitution.

The plants 15,240 and 15,245 were also crossed with 14,859, a selection

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from a third backcross of (*an.* × A 8) to A 8, lacking  $R_3^{GO}$ , homozygous for  $Y_a Y_a$ , and heterozygous for  $Ydp$ , the independent gene which reduces the expression of yellow corolla from grade 7 or 8 to about grade 4 (see Pl. 18, fig. 8). In outcrosses this gene has been shown to give a perfectly clear 1 : 1 segregation. The progeny of 14,859 with 15,240 and 15,245 would all be heterozygous  $R_3^{GO} r_3^{OO}$ , homozygous  $Y_a Y_a$ , and, if  $Ydp$  had not been segregating, should all have shown medium to intense gold. However only 50% of the progeny showed this, the remaining 50% showing a very striking rose pink coloration over the entire petal, a further character never before seen in Asiatic cottons. This type is shown in Pl. 18, fig. 12, and one of its gold sibs in Pl. 18, fig. 13. The classes were remarkably distinct, and scoring gave:

Cross	Gold	Pink
14,859 × 15,240	46	49
14,859 × 15,245	35	34

Since 14,859 was known to be heterozygous for  $Ydp$ , it was thought that this new pink might be the expression of gold on a depressed yellow corolla. To test this hypothesis, two gold and two pink selections were each crossed to A 8 (clear yellow, homozygous), and small progenies grown.

Cross	Gold	Pink	Full yellow	Low-grade yellow
19,176 = pink, × A 8	5	7	7	2
19,179 = pink, × A 8	4	6	7	5
19,217 = gold, × A 8	10	0	11	0
19,226 = gold, × A 8	6	0	18	0

The pink plants evidently carried  $Ydp$ , whilst the gold ones did not, confirming that it is the interaction between gold and  $Ydp$  which accounts for the development of pink. This however only appears to hold on a homozygous yellow corolla background. There is evidence that when yellow corolla  $Y_a$  is heterozygous, although  $Ydp$  is able to lower the expression of yellow, it does not change gold to pink. A particular example is a plant P 1822, a selection from the second backcross of (*an.* × H 10) to N 14; this plant which was spotted, must at least have been heterozygous for  $R_2^{OS}$  because of its N 14 parentage; the full spot gene  $R_2^{AS}$  not having entered this series, its spot might have been due to either  $R_2^{AO}$  from H 10 or to  $R_3^{GO}$  from *anomalum*, or to both.  $R_3^{GO}$  was definitely present because P 1822 was very faintly gold. When backcrossed again to N 14 it gave 35 spotted : 63 ghost, definitely not a duplicate gene 3 : 1 backcross ratio, thus eliminating the possibility of spotless genes in both loci. P 1822 must therefore have been  $R_2^{OS} R_2^{OS} R_3^{GO} r_3^{OO}$ . The grading of P 1822 at about 4-5 suggested that it carried

*Ydp*. There is ample evidence of this from several sources. For instance, P 1822 was crossed with N 44, a Chinese pale petal type of the constitution  $Y_a Y_a Y_b^P Y_b^P$ , and the 76 progeny were scored as follows:

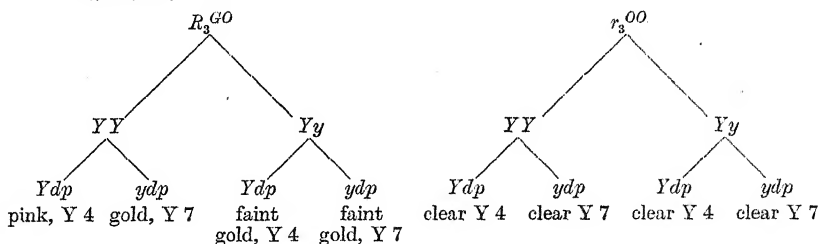
13 plants with pink (medium-intense); Y grades 4, 3, or not visible below intense pink.  
15 plants with gold (faint—very intense); Y grades 7–3, or not visible below intense gold.

48 plants with no gold or pink; Y grades 7–3.

Amongst the 48 clear yellow, grading was as follows:

Y grades				
7	6	5	4	3
9	8	5	21	5

The pink-gold segregation implied a segregation for *Ydp* acting on  $R_3^{GO}$  in the presence of a full yellow corolla potentiality. From other crosses it was already known that heterozygosity for  $Y_b^P$  does not reduce gold expression as does heterozygosity in the  $Y_a$  locus. The presence of *Ydp* was confirmed by the downward grading of the clear yellows as low as grades 4 and 3, though the expected 1:1 for full yellow:depressed yellow was somewhat obscured by the complex modifier constitution of P 1822, with three species in its pedigree. The actual segregation expected in this progeny was:



or, in a family of 76 plants, 9.5 pink, 9.5 gold, 19 faint gold, 38 no gold. Scoring for gold was not easy in those with only a faint expression, and the deviation observed may have been due either to the scoring as clear yellow of some plants which were genotypically faint gold, or to deficiency of  $R_3^{GO}$  segregates, which was also observed in the cross P 1822  $\times$  N 14,  $R_2^{OS} R_2^{OS} r_3^{OO}$ , which gave 35 red spotted:63 ghost.

It is clear that P 1822 carried both  $R_3^{GO}$  and *Ydp*, yet it showed only faint gold on depressed yellow, with only the very faintest suggestion of pink in the gold, and that mostly in the petal edge. From its N 14 parentage, P 1822 was heterozygous for  $y_a$ , and it is to this fact that the failure of *Ydp* to intensify gold to pink is attributed.

P 1822 ( $R_2^{OS} R_2^{OS} R_3^{GO} r_3^{OO}$ ,  $Y_a y_a$ , *Ydp ydp*) was also crossed with 15,240 and 15,245 ( $R_2^{OS} R_2^{OS} R_3^{GO} R_3^{GO}$ ,  $Y_a Y_a$ , *ydp ydp*). All of the

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progeny would have carried  $R_3^{GO}$ . Half of them should have been heterozygous for  $y_a$ , and therefore have shown only very faint signs of gold; of the other half homozygous for  $Y_a$ , only half should have carried  $Ydp$  and therefore shown pink, whilst the other half should have shown medium to very intense gold. Actual scoring was as follows:

	Pink	Gold				Total
		Very intense	Intense	Medium	Faint	
	17	1	2	4	6	16
Expected	11.5		11.5			23
						46

Expectation was reasonably well satisfied. The pinks were all of more or less uniform type similar to those in 14,859  $\times$  15,240. The latter were all heterozygous  $R_3^{GO} r_3^{OO}$ , yet in the family here under discussion half of the pinks should have been homozygous  $R_3^{GO} R_3^{GO}$ , implying that the maximum expression of pink is obtainable in the heterozygote. Selfed progeny from nine of the plants from P 1822  $\times$  15,240 were of particular interest in confirming these indications. Their segregation is shown in Table 10.

Table 10. *The influence of corolla colour genotype on gold expression of the  $R_3^{GO}$  allele*

Plant no. and characters	Segregation in selfed progeny										Indicated parental genotype		
	Pink					Gold							
	<i>R</i>	<i>r</i>	<i>Y<sub>a</sub></i>	<i>y<sub>a</sub></i>		Very	In- tense	Me- dium	Very faint	faint			
						in- tense							
P 2951 Y 4, pink	45	0	45	0	29	2	9	.	5	.	<i>R<sub>3</sub><sup>GO</sup></i>	<i>R<sub>3</sub><sup>GO</sup></i>	<i>Y<sub>a</sub>Y<sub>a</sub>, Ydp ydp</i>
P 2953 Y 4, pink	9	0	9	0	2	2	5	.	.	.	"	"	"
P 2958, —, pink	21	0	21	0	9	2	5	3	2	.	"	"	"
P 2955 Y 5, pink	20	7	27	0	6	2	4	2	5	1	<i>R<sub>3</sub><sup>GO</sup></i>	<i>r<sub>3</sub><sup>OO</sup></i>	<i>Y<sub>a</sub>Y<sub>a</sub>, Ydp ydp</i>
P 2954 Y 6, intense gold	15	0	15	0	.	8	.	3	4	.	<i>R<sub>3</sub><sup>GO</sup></i>	<i>R<sub>3</sub><sup>GO</sup></i>	<i>Y<sub>a</sub>Y<sub>a</sub>, ydp ydp</i>
P 2952 Y 6, faint gold	17	0	12	5	.	.	3	4	4	1	<i>R<sub>3</sub><sup>GO</sup></i>	<i>R<sub>3</sub><sup>GO</sup></i>	<i>Y<sub>a</sub>y<sub>a</sub>, ydp ydp</i>
P 2957 Y 6, faint gold	28	0	23	5	.	.	3	7	9	4	"	"	"
P 2956 Y 4, very faint gold	11	1	8	4	.	.	2	.	1	5	<i>R<sub>3</sub><sup>GO</sup></i>	<i>r<sub>3</sub><sup>OO</sup></i>	<i>Y<sub>a</sub>y<sub>a</sub>, Ydp ydp</i>
P 2959 Y 4, very faint gold	20	6	23	3	1	.	2	7	5	2	"	"	"

P 2951, P 2953, and P 2958 were, from their breeding behaviour, homozygous for  $R_3^{GO}$  and  $Y_a$ , and also carried  $Ydp$ ; their origin indicates that they could only have been heterozygous for that gene. They themselves were pink on a low grade yellow as expected (P 2958 was so intensely pink as to obscure yellow grade) and those of their progeny

which carried the depressor should also have been pink. None of these pinks, which incidentally were not very variable, were appreciably deeper than those of 14,859  $\times$  15,240, which were all heterozygous for *Ydp*. Whether any homozygous depressors occurred in these families is not known; it will be noticed that the largest of them gave 29 pink : 16 gold, much the same distorted 3 : 1 as was discussed earlier in connexion with the depressor itself. Those of their progeny lacking the depressor should have been very intense to medium gold. Some however went down to faint, which is not usual on a homozygous  $Y_a Y_a$  background. This, however, may be accounted for by the presence of other modifiers probably from the original H 10 parent of the *anomalum* hybrid. *Herbaceum* types are known, from Hutchinson's (1932*b*) work, to lack general anthocyanin intensifiers, and that H 10 lacks gold petal intensifiers in particular was shown both by the fact that the hybrid (*an.*  $\times$  H 10) (Pl. 18, fig. 6) was considerably less pink than (*an.*  $\times$  A 16) (Pl. 18, fig. 7), though both were of comparable main gene constitution, and also by the fact that 6144, an  $R_3^{GO} r_3^{OO}$  plant to which reference has already been made, gave nearly as faint gold when crossed with H 10  $Y_a Y_a$  as it did when crossed with N 14  $y_a y_a$ , though with A 16  $Y_a Y_a$  the gold grade was medium. 14,544, to which reference has also been made, gave further evidence of this. It has been shown to be of  $R_2^{OS} R_2^{OS} R_3^{GO} r_3^{OO}$  constitution, and had white corolla. On crossing with A 16 (ghost, yellow corolla) it had given 83 red-spotted segregates, with gold faint on account of heterozygosity for  $y_a$ , and 85 clear yellow ghost segregates. But on crossing with H 10 (spotless, yellow corolla) it gave 169 red-spotted progeny, on none of which gold was visible, although about half of these must have been carrying the  $R_3^{GO}$  allele. Evidently modifiers carried by H 10 further reduced the expression of this allele even beyond the very faint which would have been expected on these  $Y_a y_a$  heterozygotes. Whether these gold modifiers were the same as those which brought down the corolla grade in the backcross to H 10 by about  $\frac{1}{2}$ –1 grade below that in the family involving A 16 (Hutchinson, 1931, has shown that *herbaceum* carries yellow reducers), or whether it was the general anthocyanin reducers which were responsible, it is not possible to say. That gold petal is highly susceptible to intensification and reduction is very clear. For instance, in the homozygous  $R_2^{AO} R_2^{AO} R_3^{GO} R_3^{GO}$  progenies whose establishment has already been discussed, and from which *Ydp* was absent, the intense golds in several cases ranged over to a definite intense pink, though it was not quite the same as in the more uniformly pigmented *Ydp* type, tending to be more intense at the



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petal edge. By bagging flowers on a pink-petalled plant, it was also shown that sunlight is essential to the formation of pink, since these bagged flowers were almost pure yellow.

P 2955 in Table 10 only differed from the above three plants in being heterozygous instead of homozygous for  $R_3^{GO}$ , but was as pink as they were. P 2954 was, like the first three plants considered, homozygous for  $R_3^{GO}$  and  $Y_a$  but, since it lacked  $Ydp$ , remained intense gold. P 2952 and P 2957 were homozygous  $R_3^{GO}$ , but heterozygous  $Y_a y_a$ , and therefore only faint gold. P 2956 and P 2959 were heterozygous  $R_3^{GO} r_3^{OO}$ ,  $Y_a y_a$ . That they carried  $Ydp$  was evident, in the case of P 2959 because one of its progeny was definitely pink and in others with gold the underlying yellow was definitely of the low grade 4 depressor type, and in the case of P 2959, although in its small progeny no pinks appeared, again depressor yellow could be seen underlying some of the golds. P 2956 and P 2959 then, although they carried  $Ydp$ , were not pink but only very faint gold, because they were heterozygous for  $y_a$ .

Heterozygosity in the Chinese pale  $Y_b$  locus does not appear to affect gold expression, since in a cross of 15,240  $\times$  N 44 (only two progeny examined, of constitution  $R_3^{GO} r_3^{OO}$ ,  $Y_a Y_a$ ,  $Y_b Y_b^P$ ), the gold expression was medium to intense as in the case of heterozygous  $R_3^{GO}$  in other homozygous yellow corolla families previously examined. Heterozygosity in the *anomalum* pale  $Y_c$  locus does not reduce gold expression either; 14,854 was a selection from the third backcross of (*an.*  $\times$  A 8) to A 8, which was later found by its breeding behaviour to be heterozygous for both  $R_3^{GO}$  and  $Y_c^P$ ; it showed medium gold on a full yellow background; one of its progeny derived by selfing, no. 9992, a  $Y_c^P Y_c^P$  segregate of grade 3 yellow, was also shown to carry  $R_3^{GO}$ , but was not gold in appearance.

The factorial basis of the interaction between the main corolla colour factors and the *anomalum*  $R_3^{GO}$  allele, as deduced from these findings, is summarized in Table 11. In view of its complexity it is not surprising that at one time prospects of analysing the wide colour segregation of early generations seemed remote, especially as the various crosses behaved so differently. First of all it is worth looking back at the (*an.*  $\times$  A 16) first backcross to A 16 in Table 9. It may now be seen that this should have segregated for corolla colour, in terms of the yellow depressor, into two equal-sized groups, one grouped around grade 7 and the other around grade 4. The interspecific modifier segregation obscured this to a considerable extent. Moreover yellow grading in the red-spotted class was consistently higher than in the ghost class, and in the latter

Table 11. *The interaction between  $R_3^{GO}$  and the main factors affecting corolla colour*

Phenotype	Yellow corolla constitution				Gold petal expression	
	Genotype				$R_3^{GO}$ homozygous	$R_3^{GO}$ heterozygous
	$Y_a$ locus	$Y_b$ locus	$Y_c$ locus	$Y_{dp}$		
Yellow	Homo. $Y Y$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	(Homo. $ydp ydp$ )	Gold; very intense to int.	Gold; intense to medium
Yellow	Het. $Y Y^P$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	(Homo. $ydp ydp$ )	—	Gold; faint
Yellow	Het. $Y y$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	(Homo. $ydp ydp$ )	Gold; very faint	Gold; very faint
Pale	Rec. $Y^P Y^P$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	(Homo. $ydp ydp$ )	—	Gold; very faint or none
White	Rec. $y y$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	(Homo. $ydp ydp$ )	—	No gold; occasional faint pink edge to petal
Yellow	(Homo. $Y Y$ )	Het. $Y Y^P$	(Homo. $Y Y$ )	(Homo. $ydp ydp$ )	—	Gold; intense to medium
Yellow	(Homo. $Y Y$ )	(Homo. $Y Y$ )	Het. $Y Y^P$	(Homo. $ydp ydp$ )	—	Gold; intense to medium
Pale	(Homo. $Y Y$ )	(Homo. $Y Y$ )	Rec. $Y^P Y^P$	(Homo. $ydp ydp$ )	—	No gold
Yellow	Homo. $Y Y$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	Het. $Ydp ydp$	Pink	Pink
depressed	Het. $Y y$	(Homo. $Y Y$ )	(Homo. $Y Y$ )	Het. $Ydy ydp$	Gold; very faint	Gold; very faint
Yellow						
depressed						

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there was far less clear distinction into the two expected groups. This was partly due to the fact that the biochemical interaction between flavone and anthocyanin appears to work in two directions—thus although lowering of yellow grade leads to intensification of gold to pink, the development of these two latter colours can also lower yellow grade to some extent, and the levels at which development of yellow and pink are ultimately established are the result of the balance between these two tendencies. The higher grading of the ghosts may also partly be due to a subjective tendency in scoring, yellow appearing brighter in the absence of gold or pink. Within the red-spotted class it will be seen that most of the high grade yellows were gold and the depressed yellows pink. In a few of the high grade yellows gold was intensified to pink by modifiers other than the yellow depressor; such types were usually phenotypically different, most of the pink being concentrated towards the petal edge instead of evenly distributed over the petal as in the case of the depressor type.

The hybrid *an.* × A 8 (Pl. 18, fig. 5) showed considerably less pink than *an.* × A 16 (Pl. 18, fig. 7) being really only medium gold. Since in constitution it was heterozygous for  $R_3^{GO}$ , homozygous  $Y_a$ , and carried  $Ydp$ , exactly as the A 16 hybrid, it should have been rather more pink than it was. This can only be attributed to the absence of pink intensifiers in both A 8 and *anomalum*, since plants of the same *main* gene constitution as their  $F_1$  were later synthesized, using 15,240 as the immediate source of the  $R_3^{GO}$  allele, and 14,859 as the source of  $Ydp$ , which gave the pinks which have already been discussed (Pl. 18, fig. 12). In this series intensifiers derived from A 16 (by way of 15,240) came into play, the presence of which accounted for the suitability of backcrosses to A 16 for the study of gold. For this reason A 16 has been regarded as the "standard" basis for discussion of gold expression. Only small backcrosses of (*an.* × A 8) to A 8 were grown, and since there was no specific "tag" such as the spot segregation which occurred in the A 16 backcrosses to assist with gold scoring, the latter was not seriously dealt with in these lines. Large backcrosses of (*an.* × A 8) to N 14 and H 10 were grown, but heterozygosity for  $y_a$  in the former, and the absence of intensifiers in the latter, rendered these families also unsuitable for observation of gold.

The hybrid *an.* × H 10 (Pl. 18, fig. 6), showed slightly less gold than *an.* × A 8; this again was comparable in main gene constitution with the hybrid *an.* × A 16, and it can only therefore be assumed that H 10 lacks gold intensifiers. Other indications of this have already been discussed. H 10 cannot however lack intensifiers so completely as does *anomalum*

itself, as in a small first backcross to H 10 those plants which showed gold were of medium grade, more intense than the  $F_1$ .

With regard to *anomalum* itself, the corolla of which is very pale cream, almost white, there is only the faintest suggestion of pink over the petal (Pl. 18, fig. 4). This is no doubt particularly due to its low-grade corolla colour. The appearance of gold and pink in crosses between *anomalum* and *arboreum* or *herbaceum* is a most characteristic and unexpected feature of these hybrids. As far as the modifier level of *anomalum* is concerned, there is not a great deal of direct evidence available. The fact that there is a general tendency for gold and pink to become accentuated in passing from the  $F_1$  hybrid to later backcross generations to the cultivated species, suggests that the hybrid must itself have been brought down by the low level of *anomalum*. Only very few moderately large families of backcrosses to *anomalum* have been grown. In one, a selfing of a second backcross of H 10 (yellow corolla), there was a segregation 53 yellow corolla (4-5-6 of depressed type) : 27 cream of *anomalum* type (no doubt = 3 : 1). Amongst the yellows there were no real pinks, only golds ranging from intense to very faint, and some were apparently clear yellow, yet all must have carried  $R_3^{GO}$  and some at least the depressor from *anomalum*, and all would have been homozygous  $Y_a Y_a$ ;  $Y_c Y_c^P$  was segregating, but in the heterozygous condition this locus has been shown not to affect gold expression. The inference is therefore that the modifier level is not high enough in *anomalum* to result in the production of pink where it would otherwise be expected, nor was the gold as intense on the average as it would have been in a family of similar constitution in an *arboreum* genotype. That there was also an introduction of pink intensifiers normally absent from *anomalum* was evident from the fact that some of the pale petal segregates showed a definite pinkish tinge, not at all intense, but much more marked than in *anomalum* itself. The evidence on the whole points to *anomalum* lacking the modifiers which intensify gold and pink, as well as the main gene for flavone development essential to the full expression of the  $R_3^{GO}$  allele.

#### (6) *Spot size modifiers*

In New World cottons there is an exceedingly wide range in size of petal spot, from only a few pigmented cells to a large intensely pigmented area. In segregating progenies the range was such that Harland (1929a, Pl. IX) found it necessary in scoring to make use of a graduated scale of 22 classes, in which grade 1 was the smallest with only a trace of pigment, and grade 22 the largest. In cultivated Asiatic cottons, on the

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other hand, there is much less variation in size and intensity of spot where present, so that Hutchinson (1932*b*) in his detailed study of anthocyanin found a graduated scale quite unnecessary, though in crosses between *arboreum* and *herbaceum* there was some indication that in one *herbaceum* type at least (H 10, spotless) the spot intensity genotype was at a slightly lower level than in most other strains, and size and intensity of spot are to some extent correlated. Practically all *arboreum* and most *herbaceum* types have a spot of about size 17–19 on Harland's scale, though many *herbaceum* strains, especially of varieties *typicum* and *africanum*, have a slightly smaller spot. The A 8 strain used in this study has a rather larger spot, which is quite at the top end of the range of Asiatic spots. The A16 ghost type has a rather small ghost area, which is right at the lower end of the normal range in Asiatic spot sizes. Recently Hutchinson & Ghose (1937*b*) have described an uncommon small spot type from *arboreum*, about one-eighth of the usual size, which they attribute primarily to a petal spot reducer (*Sr*). In the New World cottons Harland did not find it possible to identify particular genes affecting spot size, but different strains and species differed radically in their entire spot size genotype. The discovery by Hutchinson & Ghose of a particular and very rare size reducer does not affect the general situation that the spot size genotype in *arboreum* and *herbaceum* is remarkably uniform in contrast to the position in New World cottons.

The spot of *G. anomalum* is also of the normal size common in the cultivated Asiatic species, but in its interspecific hybrids the spot is very markedly enlarged (Pl. 18, figs. 5–7), and usually has a very characteristic streaked edge, whereas in *anomalum* and the Asiatic cottons it is clearly delimited. In progenies derived from these hybrids there was a very wide range in spot size, extending even beyond that in Harland's scale, and as the latter spots were of rather different shape from those under examination, a new scale of grades was devised. The new series contained fifteen classes, of which 1 was the smallest, similar to Harland's grade 1, and 15 the largest. It has not been thought necessary to reproduce this scale here, but in order to give some indication of the range, the size of some of the spots shown in Pl. 18 is indicated in the legend. Usually three flowers were graded on each plant, and the mean taken.

Hutchinson (1932*b*) has shown that H 10, the *herbaceum* spotless type, lacks anthocyanin intensifiers, so that the red spot area in ghost-spotless compounds is smaller than the underlying "ghost" which appears as a clear white marginal area around the pigmented spot, which is of lower intensity than most full spot types (Pl. 18, fig. 14). In practice it is

not easy to differentiate between spot size genes and spot intensity modifiers. In general larger spots are more deeply pigmented than smaller ones, and large faint spots or small deeply pigmented ones are not encountered, though for each size of spot there is a slight range in intensity. In a small  $F_2$  between H10 and an *arboreum* ghost, Hutchinson found some range in size and intensity of the spot compound. From his description it appears that his extremes would correspond with my grades 3 and 8, and this has been confirmed in a repeat  $F_2$  of similar type. Omitting Hutchinson & Ghose's "small spot type" it may be summarized that Asiatic spots normally range from size 10 to 12, very occasionally to 13; whilst in some interspecific hybrids the spot compound may range down as low as 3. This latter is of course a very exceptional case amongst the cultivated Asiatic cottons. In the *anomalum* interspecific hybrids and their progeny a very much wider segregation was encountered, right from complete extinction of the spot by modifiers up to size 15 and occasionally even slightly above this. Some particular examples will be discussed.

*G. anomalum* spot is graded as size 12; A16 is size 10; the hybrid has a considerably larger spot than either of its parents, of grade 13-14. In the first backcross to A 16, segregating 1 red spot  $R_2^{OS} R_2^{OS} R_3^{GO} r_3^{OO}$  : 1 ghost spot  $R_2^{OS} R_2^{OS} r_3^{OO} r_3^{OO}$ , size was scored in both classes, and the wide range encountered is shown in the following frequency table:

Spot size													
	15	14	13	12	11	10	9	8	7	6	5	4	3
Red spot	10	31	20	21	20	8	8	4	5	.	.	.	3
Ghost	.	5	18	20	23	10	14	5	3	.	.	1	1
													5
													105
													12.08
													10.91

$$d=1.17; \quad t=4.47; \quad P \text{ very small.}$$

This very wide range in size segregation shows that the size genotypes of the two parental species must be very differently constituted, though their end effects are much the same. On the average, spot on the red-spotted types was a little more than one grade larger than the ghost on ghost-spotted types, and the difference was highly significant. The tendency persisted into some lines of later backcrosses. In the second backcross to A.16 only small progenies were grown, so sufficiently large classes to make the comparison in size worth while are not available. From two of the second backcross lines seven plants were again backcrossed and selfed. These progenies had very distinct size ranges, as is shown below, giving very definite evidence that size is genotypically controlled.

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Plant no. and spot size		Spot size of progeny																			
		B.C. progeny									Selfed progeny										
		14	13	12	11	10	9	8	7	6	14	13	12	11	10	9	8	7	6	5	4
P 1325	Size 9	Red spot	.	.	.	1	6	2	.	.	.	.	.	2	4	4	1	1	.	1	.
		Ghost	.	.	.	.	2	1	2	.	1	.	.	1	1	2	.	2	1	.	1
P 1326	Size 9	Red spot	.	.	.	.	.	1	1	.	.	.	.	.	.	.	1	1	2	.	.
		Ghost	.	.	.	1	.	1	.	.	.	.	.	.	1	.	.	.	.	.	.
P 1327	Size 10	Red spot	1	2	3	1	1	2	1	.	.	.	.	.	1	.	1	.	1	.	.
		Ghost	.	.	4	1	2	.	4	1	.	1	.	.	1	.	.	1	.	.	.
P 1338	Size 11	Red spot	.	3	.	3	1	.	.	1	.	1	1	2	.	.	.	.	.	.	.
		Ghost	.	2	2	3	1	4	3	1	.	.	.	1	1	.	2	.	.	.	.
P 1339	Size 12	Red spot	1	3	.	2	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.
		Ghost	.	2	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
P 1340	Size 12	Red spot	.	.	.	3	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ghost	.	.	.	.	5	4	3	2	.	.	.	1	1	.	.	.	.	.	.
P 1341	Size 11	Red spot	.	2	2	1	.	.	.	.	.	2	1	4	3	.	.	1	.	.	.
		Ghost	.	.	1	3	1	1	.	.	.	.	.	1	.	.	1	.	.	.	.

The tendency for red-spotted types to grade larger than ghosts is brought out more clearly when these seven third backcross progenies are considered in total:

	Spot size										
	14	13	12	11	10	9	8	7	6	Total	Mean
Red spot	2	10	5	11	9	6	2	.	1	46	10.97
Ghost	.	4	7	9	12	10	13	4	1	60	9.71

$$d=1.26; \quad t=3.66; \quad P \text{ very small.}$$

Three selections from these third backcrosses which were selfed are also interesting in showing the very distinct size genotypes which can be established:

Plant no. and spot size		Spot size of progeny												Total
		14	13	12	11	10	9	8	7	6	5	4	3	2
15,094	Size 14	3	13	14	8	8	7	9	3	1	.	.	.	66
15,095	Size 8	.	.	.	.	.	.	7	10	9	.	1	2	30
15,099	Size 8	.	.	.	.	.	4	5	3	3	3	1	2	21

The first of these families ranges considerably higher than an Asiatic family normally does, while both of the other two families contain spot reducers. The latter families are too small to make the size distinction between spot and ghost worth while, but in the large family the mean size for red-spot plants was 11.06, and that for ghosts 10.09, again a significant difference (0.97,  $t=2.14$ ,  $P=0.05-0.02$ ). The data strongly suggest that  $R_3^{GO}$  is associated with a slightly larger size effect.

There is further evidence on this point from the first backcross of the same hybrid (*an.*  $\times$  A 16) to H 10. All progeny were red-spotted, but some had gold petal and others were clear yellow. The expected segregation was 1:1 for the two genotypes  $R_2^{OS} R_2^{AO} R_3^{GO} r_3^{OO}$  and  $R_2^{OS} R_2^{AO} r_3^{OO} r_3^{OO}$ , the former gold and the latter clear yellow. Gold petal was not easy to

score, as a large number were very faint gold on account of the influence of H 10; amongst the plants adequately scored there was a deficiency of gold segregates, and in the absence of any definite evidence as to the cause, the most plausible explanation is that some genotypic golds were scored as clear yellow. Notwithstanding this there was still a significant difference in spot size between the two classes:

	Spot size									Total	Mean
	15	14	13	12	11	10	9	8	7		
Gold	11	13	14	5	3	3	2	.	1	52	13.01
No gold	6	27	17	9	10	7	8	7	5	96	11.87

$d=1.14$ ;  $t=3.04$ ;  $P$  very small.

In this backcross an attempt was made to differentiate full spot from spot compounds, as it was still thought that the *anomalum* duplicate was a full spot. About 50% of the plants up to grade 11, and only 14% of those above, were scored as spot/ghost, the others as full spot. It is now known that this was of no significance to the main gene segregation, and was only an expression of the tendency for large spot to be more diffuse at their edges, thus obscuring any white margin which is more easily seen beneath a smaller fainter pigmentation area. A similar case occurred in the family 14,544 ( $R_2^{OS} R_2^{OS} R_3^{GO} r_3^{OO}$ )  $\times$  H 10 ( $R_2^{AO} r_3^{OO}$ ), in which it was not possible to see gold at all, as all plants were heterozygous for  $y_a$  as well as carrying the H 10 anthocyanin reducing genotype. 85 of the plants were scored as full spot phenotypically, and 84 as indefinite spot/ghost, though there was very little size segregation in the family. The similarity to the last case suggests that the full spot phenotypes probably carried  $R_3^{GO}$ , and that the slightly less intense spot types were the result of the  $R_2^{OS} R_2^{AO}$  compound.

P 2381 and P 2386, two plants derived by the intercrossing of a red spot selection from the second backcross to A 16, with H 10, have already been discussed. It has been shown that their constitution must have been  $R_2^{OS} R_2^{AO} R_3^{GO} r_3^{OO}$ , and that on backcrossing to H 10  $R_2^{AO} r_3^{OO}$ , four genotypes were obtained, in approximately equal numbers as expected—red spot, gold; red spot, no gold; spotless, gold; spotless, no gold. There was however an amazing difference in the spot-size range in the progenies from these two plants; those derived from P 2381 ranged right up to grade 15, many being of the type with streaked diffuse edges. Derivatives of P 2386 had ordinary Asiatic spot size not above 12–13, with edges fairly clearly defined and not streaked. Unfortunately on account of the less spectacular range in the latter family spot size was not



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graded there, but this was carried out in the former family, again clearly showing the association of larger size with  $R_3^{GO}$ :

	Spot size					Total	Mean
	15	14	13	12	11		
Gold	7	29	3	.	1	40	14.02
No gold	1	8	12	1	1	23	13.30

$d=0.72$ ;  $t=3.69$ ;  $P$  very small.

There is no positive evidence that it is possible to separate this particular size effect from the gene  $R_3^{GO}$ , and therefore no justification for postulating that it is due to the action of an independent size modifier on the same chromosome.

Other material gave evidence of the association of a size effect with the  $R_2$  locus in *anomalum* also. This effect was somewhat greater than that associated with  $R_3$ , and it was possible in certain lines to separate it from the *anomalum* ghost, indicating that it was due to a distinct gene. The  $F_1$  of *anomalum* (spot size 12)  $\times$  A 8 (spot size 13) showed the usual large spot characteristic of these hybrids, of size 13-15 (Pl. 18, fig. 5). Backcrosses to the ghost types N 14 and T 3, segregating 3 red spot : 1 ghost, showed again the very wide range from rather larger than 15 down to 4. Although the difference in size between the red-spotted types (which included both full spot  $R_2^{AS}$  and the compound  $R_2^{OS} R_3^{GO}$  types) and the ghost types was not significant, it is of some importance to the argument to indicate that in both families the ghost spots tended to grade a little larger than the red-spotted class.

Cross		Spot size												Not graded	Total	Mean
		15	14	13	12	11	10	9	8	7	6	5	4			
(an. $\times$ A 8) $\times$ N 14	Red spot	8	17	24	13	28	16	11	7	3	1	1	.	45	174	11.48
	Ghost	1	5	8	3	4	1	1	.	1	.	.	1	3	28	11.96
$d=0.48$ ; $t=0.99$ ; $P=0.3$ .																
(an. $\times$ A 8) $\times$ T 3	Red spot	6	16	25	11	12	10	12	5	1	2	3	.	.	103	11.50
	Ghost	.	11	14	2	.	3	.	4	1	.	.	.	.	35	12.25
$d=0.75$ ; $t=1.62$ ; $P=0.1$ .																

The hybrid (an.  $\times$  A 8) was also backcrossed with H 10, again giving a very wide size segregation.

Spot size												Total
15	14	13	12	11	10	9	8	7	6	5		
66	117	134	73	53	51	22	10	3	.	1		530

It has already been demonstrated that two selections from this back-cross, nos. 11,703 and 11,757, of size 15 and 13 respectively, must have been of constitution  $R_2^{OS}$  (from *anomalum*)  $R_2^{AO}$  (from H 10)  $r_3^{OO} r_3^{OO}$ .

These plants when crossed with ghost types ( $R_2^{OS}$ ) gave 1 : 1 segregations for the compound spot  $R_2^{AO} R_2^{OS}$  and ghost  $R_2^{OS} R_2^{OS}$ , and the ghosts were found to grade consistently higher than the compounds:

Cross		Spot size														Not graded	Total	Mean
		16	15	14	13	12	11	10	9	8	7	6	5	4	3			
11, 703 × N 14	Red spot	.	1	1	11	9	7	8	2	1	1	1	.	.	.	.	42	.
	Ghost	2	4	4	6	2	.	.	.	.	.	.	.	.	.	2	20	.
11,703 × T 3	Red spot	.	.	1	12	15	19	15	15	7	5	1	2	.	.	.	93	.
	Ghost	3	7	19	17	6	6	1	1	2	.	.	.	.	.	.	62	.
11,703 × T 3 (2nd sowing)	Red spot	.	.	1	2	9	13	13	16	15	12	3	1	.	.	.	85	.
	Ghost	.	2	16	25	13	5	.	4	1	1	.	.	.	.	.	67	.
11,703 Total	Red spot	.	1	3	25	33	39	36	33	23	18	5	3	.	1	.	220	10.09
	Ghost	5	13	39	48	21	11	1	5	3	1	.	.	.	.	2	149	12.95
$d=2.86$ ; $t=13.84$ ; $P$ very small.																		
11,757 × N 14	Red spot	.	1	3	9	3	5	2	1	.	.	.	.	.	.	1	25	12.25
	Ghost	.	4	13	3	.	1	.	.	.	.	.	.	.	.	.	21	13.90
$d=1.65$ ; $t=4.44$ ; $P$ very small.																		

This is obviously not the result of a subjective tendency in scoring, as was at one time thought might be the case, since in the series previously considered ghosts were found to be smaller than red spots. It will of course be realized that although the phenotypes being contrasted are the same in both series, the genotypes are not. The essential point of significance is that in this series the  $R_2^{OS}$  allele under consideration is of *anomalum* origin, and this appears to be associated with large size. Of course in the first two families considered in this series, (*an.* × A 8) backcrossed to N 14 and to T 3, the *anomalum*  $R_2^{OS}$  allele was present in *all* the ghost segregates, and in *some* of the red-spotted group. The expected size segregation would have been 1 large compound spot + 2 ordinary-sized full spot (these two genotypes not separable phenotypically) against 1 large ghost. The size distinction tendency was present, but naturally the occurrence of the large-sized class in each of the two main phenotypes tended to obscure the contrast. In the derivatives of 11,703 and 11,757 the two classes compared were ghost containing  $R_2^{OS}$ , both from *anomalum* and from the backcross parent, and compound spot in which ghost came from the latter only, so that the size distinction associated with the introduced gene was quite clear.

These same two plants have been crossed with H 10, segregating 1 compound spot : 1 spotless. The former graded as follows:

Cross	Spot size						Total	Mean
	15	14	13	12	11	10		
11,703 × H 10	5	12	14	2	2	.	35	13.46
11,757 × H 10	6	50	66	8	6	3	139	13.24

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It will be seen that these spot compounds, which carried  $R_2^{OS}$  of *anomalum* origin, graded larger than those in the N 14 and T 3 backcrosses, which contained  $R_2^{OS}$  from the latter types. It was also recorded during scoring that they were considerably more intense than those in the latter backcrosses. In size they corresponded with the *ghost* segregates in the N 14 and T 3 backcrosses, as would be expected if large size is associated with the *anomalum* allele. It is not a question of the difference in size of the spot compounds in the two backcrosses being due to the genotype contributed by the *arboreum* and *herbaceum* backcross parents respectively. If that had been the explanation, the compound would have been smaller in the H 10 series, whereas the reverse was the case. This size effect is obviously of considerable magnitude. The striking differences in spot size between sib-progenies which have already been demonstrated in other crosses (e.g. 15,094 progeny *versus* those from 15,095 and 15,099; P 2381 *versus* P 2386) strongly suggested the existence of a single gene of some considerable effect. It is also significant that selections which were known to carry  $R_2^{OS}$  from *anomalum*—P 1791, P 1801, P 1818, 11,319, 11,703 and 11,757—all also showed the size effect.

In particular lines however this effect has become dissociated from the *anomalum* allele. Some selections from the third backcross of (*an.*  $\times$  A 8) to A 8 were selfed. The progenies showed different size ranges, and two which carried  $R_2^{OS}$  (15:1 segregation), which must have originated from *anomalum* because A 8, the only other type in their progeny, is homozygous  $R_2^{AS}$ , were segregating up to size 15. Some of the *ghosts* in these families however were as low as 7. A red-spot selection, no. 9992, from one of the segregating selfed progenies, of size 13, at the top of the Asiatic range but not beyond, was found to carry  $R_2^{OS}$ . It was crossed with the *arboreum* *ghost* type A 16, and backcrossed to 9992, and all five of the families grown, as previously described, segregated red spot: *ghosts* in 7:1 or 15:1 ratios. These *ghost* segregates must have contained at least one  $R_2^{OS}$  derived from 9992 and therefore from *anomalum*, yet on all of them as well as on the red-spotted types the spot was of perfectly normal Asiatic size. It cannot be allowed that the *ghosts* were small on account of their modifier background, because this was predominantly *arboreum*. Evidence will be presented later to show that dominance is in the direction of large size, so that the *anomalum* spot must have become dissociated in this line from its earlier size effect, which implies that in this case the effect must be due to a fairly closely linked but not inseparable gene.

The  $F_1$  *an.*  $\times$  H 10 spotless also showed a large spot of size 14-15, and the first backcross to H 10 gave the usual wide range:

Spot size						Total red spot	Spotless
14	13	12	11 ... 4	Not graded			
8	7	10	2	1	1	29	28

Only small second and third backcrosses were grown, and it is not worth discussing the results in detail. The range was similar to that in the first backcross. One of the second backcross plants, P 1898, with red-spot size 14-15, was selfed. From its origin and phenotype it must have carried  $R_2^{40}$  from H 10, and  $R_2^{OS}$  from *anomalum*. Its behaviour showed that it did not carry  $R_3^{GO}$ . This locus may therefore be ignored. A backcross to A 16 ghost gave:

Spot size								
	15	14	13	12	11	10	9	8
Red spot	.	.	1	2	1	.	.	1
Ghost	1	3	1	.	.	.	.	.

On backcrossing to H 10 it threw:

Red spot, size			Spotless
15	14	13	
1	1	2	4

Although the progenies were small, the larger size and greater intensity of the red-spot compounds in the H 10 backcross was in striking contrast to the usual situation where comparable compounds in the *herbaceum* genotype usually grade lower than in an *arboreum* genotype. Their only distinction was that whereas the compound in the A16 backcross carried ghost from A 16, that in the H 10 backcross derived its ghost from *anomalum*. The same tendency for ghost spots from *anomalum* to range higher was also observable in a backcross of the same plant P 1898 to 14,350, a ghost genotype which carried minus modifiers so that it appeared spotless, and whose origin and behaviour will be discussed later.

Spot size								
	15	14	13	12	11	10	9	8
Red spot	.	.	4	3	2	.	.	1
Ghost	3	7	2	.	.	.	.	.

The fact that the ghost in this series still retained its large size into the third backcrosses into Asiatic types suggests that the accompanying size gene must be fairly closely linked with the  $R_2$  locus.

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A large ghost segregate, 15,305, of size 14, selected from a selfed progeny of P 1898, was crossed with the following main spot types:

A 8—red full spot,  $R_2^{4S}$ —itself large in the Asiatic range, size 13 (Pl. 18, fig. 1).

A 16—ghost,  $R_2^{OS}$ —low in the Asiatic range, size 10 (Pl. 18, fig. 3).

H 10—spotless,  $R_2^{AO}$ —A 16  $\times$  H 10 gives a small red-spot compound, about size 7 (Pl. 18, fig. 14).

15,240—red-spotted compound,  $R_2^{OS} R_2^{OS} R_2^{GO} R_3^{GO}$ —the synthesized *anomalum* compound on A 16 background, size 12 (Pl. 18, fig. 9).

The way in which 15,305 raised all these spot types is shown below:

Cross	Progeny	Spot size				
		15	14	13½	13	12
15,305 $\times$ A 8	Red spots	23	17	.	.	(Pl. 18, fig. 16)
15,305 $\times$ A 16	Ghosts	.	6	6	3	1 (Pl. 18, fig. 17)
15,305 $\times$ H 10	Red-spot compounds	.	7	4	1	1 (Pl. 18, fig. 18 is of this type)
15,305 $\times$ 15,240	Red-spot compounds	3	25	.	8	1 (Pl. 18, fig. 7 is of similar genotype and phenotype)

15,305, which is homozygous for the ghost allele  $R_2^{OS}$  from *anomalum*, is also apparently homozygous for the associated large spot gene, and dominance is obviously strongly in the direction of large size. All these large spot types also had the diffuse edges which are such a characteristic feature of the large spots of the *anomalum*  $\times$  Asiatic  $F_1$  hybrids. It is evidently to this particular spot size modifier associated with  $R_2$  that this effect is due. The size modifier is obviously not specific to any particular allele, but affects ghost, full spot, and red-spot compounds, both those due to complementary alleles at the same locus, and those due to complementary genes in duplicate loci.

The *an.*  $\times$  H 10 hybrid was also backcrossed repeatedly to N 14 (ghost spot, white corolla), and a very interesting situation arose in this material, in which it was possible to select in the reverse direction for small-size modifiers. In the first backcross, segregating 3 spot : 1 ghost, size ranged from 15 to 5. One of the ghost segregates was again backcrossed, giving 3 yellow corolla ghosts and 9 white corolla segregates, which presumably also carried ghost. These plants were selfed, but since ghost can only be seen on yellow corolla and not on a white background, attention will be confined to the yellow-petalled progeny of the three yellow-petalled second backcross plants, P 1850, P 1852, and P 1853. Since these plants were ghost spot, they could not have been carrying the spotless allele  $R_2^{AO}$  or they would have shown the red spot-compound, but nevertheless some completely spotless types appeared amongst their yellow-petalled progeny. As this result was so unexpected the three parent ghosts were

again selfed the following season, with the same result. The total scoring is summarized below:

	Yellow corolla										White corolla				
	Ghost definite, large		Ghost definite, small			Ghost indistinct			Definite spotless						
P 1850 selfed	30		8			2			5		16				
P 1852 selfed	4		.			1			3		3				
	Yellow corolla Size of ghost										White corolla				
	13	12	11	10	9	8	7	6	5	4	3	2	1	0	
P 1853 selfed	4	14	9	9	10	8	5	2	.	1	.	.	.	6	40

In each of these families ghost spot size ranged right down to the point of extinction without any real break in the distribution. In the progeny of P 1850 two plants were graded as indistinct ghost, and also one in the progeny of P 1852, and two (sizes 6 and 7 respectively) in the progeny of P 1853. These indistinct types need special description. In the true spotless type  $R_2^{40}$  (H 10) there is a tendency for the yellow pigment of the corolla to be slightly more intense right towards the base of the petal, in the area usually occupied by the spot when present. In ghost and red-spot types this basal intensification does not occur. The spotless types in these progenies were similar to the usual type, with yellow slightly intensified at the base of the petal. In the distinct ghost segregates the ghost area was perfectly white, and the upper yellow portion of the corolla uniformly pigmented. The intermediate types scored as "indistinct ghost" had the yellow pigment deepening towards the base of the petal, and a small area right at the base of slightly paler yellow, giving the appearance of a pale yellow small ghost, instead of the usually clear white one.

One of the spotless plants from the progeny of P 1852, no. 14,350, was further investigated. To confirm that it was not a new type of spotless recessive to ghost but not complementary with it, it was crossed with H 10 spotless. All the progeny were red spotted, showing that genotypically 14,350 was an extinguished ghost. In addition 14,350 was crossed with A 16 (ghost, size 10), giving ghosts only, and with A 8 (full spot, size 13), and was also selfed. Spot sizes in these four progenies were as follows:

Cross	Phenotype of progeny	Yellow corolla Spot size													White corolla	
		13	12	11	10	9	8	7	6	5	4	3	2	1	0	
14,350 × H 10	Red spot	8	13	13	8	9	7	2	2	2	.	.	.	.	.	.
14,350 × A 16	Ghost	7	8	5	5	3	2	3	.	1	1	1	1	.	.	.
14,350 × A 8	Red spot	14	4	2	.	.	.	.	.	.	.	1	3	3	28	25
14,350 selfed	Ghost	.	.	.	.	1	1	1	7	3	2	1	3	3	28	25

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The selfed progeny threw only a few small ghosts, all of which were of the indistinct type, and the majority of the yellow-petalled segregates were completely spotless. A constant extinguished ghost line has not been established, but no doubt it could be from this material. In the family 14,350  $\times$  A 16, in which the normal size complement was brought in by the latter parent, the general size level was of course raised. All the larger ghosts were perfectly clear, but all below size 8 were of the indistinct type. In 14,350  $\times$  H 10 there was the usual correlation between size and intensity of red pigment. Many of the progeny, both large and small-spotted types, had no sign of any white surround. Some of the fainter ones without white margin were very flecked and indefinite (Pl. 18, fig. 15), and highly suggestive of many New World spot types, which are in general very distinct from the intense well-defined spot characteristic of Asiatic cottons. These families again give evidence on the direction of dominance of large spot modifiers, the majority of spots in the outcross progeny of 14,350 coming well within the Asiatic range. They afford an interesting comparison with the similar outcrosses involving 15,305, the large ghost, which have been discussed above. It seems likely that several modifiers are concerned in 14,350. They apparently do not include the spot size reducer *Sr* of Hutchinson & Ghose (1937*b*) since the latter was dominant in  $F_1$  crosses with normal-sized Asiatic spots<sup>1</sup>.

In addition to studying the association of spot size with the main anthocyanin loci, that with the genes  $L^A$ ,  $p_a$ , and *Ne* from *anomalum* has also been observed in some families. The data were not very extensive, and no definite conclusions could be drawn, other than that there were no striking effects associated with these genes. In one series of backcrosses derived from (*an.*  $\times$  A 8) there was some suggestion of a small difference in spot size associated with the *L* chromosome:

Cross	Class	<i>n</i>	Mean spot size	<i>d</i>	<i>t</i>	<i>P</i>
( <i>an.</i> $\times$ A 8) $\times$ H 10	$L^L$	281	12.36	+0.43	2.75	Very small
	$L^A$	266	12.79			
( <i>an.</i> $\times$ A 8) $\times$ N 14	$L^L$	77	11.62	-0.22	0.61	0.5
	$L^A$	77	11.40			
( <i>an.</i> $\times$ A 8) $\times$ T 3	$L^L$	69	11.73	-0.07	0.17	0.9
	$L^A$	69	11.66			

The results were, however, not consistent, though this may be due to a difference in this respect between the *arboreum* and *herbaceum* backcross

<sup>1</sup> That *Sr* is not one of the small size genes concerned in 14,350 has been confirmed since this manuscript was sent to press. Amongst a total of 70  $F_2$  progeny derived from the cross (reduced spot  $\times$  14,350), nine plants had petal spot of full normal size.

parents. Indication of a similar difference between the two cultivated species was also found in lint length genotype (see § XI).

(7) *Linkage tests with  $R_3$*

The discovery of the gold spotless allele provides a new locus for linkage investigation in the cultivated Asiatic species. Information has been collected in the course of the backcrossing reported in this paper, and after  $R_3^{GO}$  had been established in the *arboreum* genotype a number of crosses were designed specifically for linkage testing. The available data have been summarized in Table 12. All crosses were of the  $R_3X-r_3x$

Table 12. *Two-factor segregations involving  $R_3$*

Factor	Family	Type	$R_3X$	$R_3x$	$r_3X$	$r_3x$	$T$	$\chi^2L$	$P$
$L$	( <i>an.</i> $\times$ N 14) $\times$ N 14	B.C., $F_1 \varnothing$	39	45	30	39	153	0.13	0.7
	( <i>an.</i> $\times$ N 14) $\times$ N 5	B.C., $F_1 \sigma$	22	40	10	29	101	1.07	0.3
	(15,099 $\times$ P 2774) $\times$ T 17	B.C., $F_1 \varnothing$	45	46	45	43	179	0.05	0.8
	15,240 $\times$ N 5 or N 14	$F_2$	297	117	84	27	525	0.68	0.5
$Lc_1$	(P 2406 $\times$ T 1) $\times$ N 14	B.C., $F_1 \varnothing$	34	22	24	42	122	7.20	Very small
$H_a$	(P 1338 $\times$ 429) $\times$ T 1	B.C., $F_1 \varnothing$	164	188	196	169	717	3.62	0.06
$Ne$	( <i>an.</i> $\times$ N 14) $\times$ N 14	B.C., $F_1 \varnothing$	42	46	33	38	159	0.02	0.9
	( <i>an.</i> $\times$ N 14) $\times$ N 5	B.C., $F_1 \sigma$	40	22	22	18	102	0.92	0.7
	15,240 $\times$ N 14 or N 5	$F_2$	357	98	101	33	589	0.57	0.5
	(15,099 $\times$ T 4) $\times$ T 4	B.C., $F_1 \varnothing$	20	17	24	21	82	0.004	0.95
$P_a$	(P 1327 $\times$ P 2375) $\times$ T 3	B.C., $F_1 \varnothing$	51	71	50	61	233	0.25	0.7
$Pdy$	(15,099 $\times$ P 2774) $\times$ T 17	B.C., $F_1 \varnothing$	51	41	43	46	181	0.92	0.3
$Y_a$	( <i>an.</i> $\times$ N 14) $\times$ N 14	B.C., $F_1 \varnothing$	40	48	37	35	160	0.56	0.5
	( <i>an.</i> $\times$ N 14) $\times$ N 5	B.C., $F_1 \sigma$	30	30	20	19	99	0.01	0.9
	(P 1338 $\times$ 429) $\times$ T 1	B.C., $F_1 \varnothing$	164	154	158	162	638	0.31	0.5
	(15,099 $\times$ T 4) $\times$ T 4	B.C., $F_1 \varnothing$	17	20	26	19	82	1.14	0.3
	(15,099 $\times$ P 2774) $\times$ T 17	B.C., $F_1 \varnothing$	51	41	38	51	181	2.94	0.1
	15,240 $\times$ N 14 or N 5	$F_2$	318	105	104	30	557	0.33	0.5
$Y_c$	(9992 $\times$ A 16) $\times$ 9992	$R_{15:1}, Y_{1:1}$	83	45	7	1	136	1.73	0.2
	(9992 $\times$ A 16) $\times$ 9992	$R_{7:13}, Y_{1:1}$	109	89	13	14	225	0.46	0.5

type except in the case of the  $Lc_1$  test. These figures show that  $R_3$  is independent of the three linkage groups—leaf shape—lint colour,  $L-Lc_1$  ( $L-K$  group of Hutchinson, 1934); lintless—lint colour,  $H_a-Lc_2$  (Silow, unpublished); and nectaries—pale pollen,  $Ne-P_b$  (Silow, unpublished).  $Lc_1$  and  $R_3$  gave a considerable deviation, but the association was the reverse of that in the parents. The family was fairly small, and no significance can be attached to this observation.  $\chi^2L$  for the  $R_3H_a$  segregation was only just below the conventional limit of significance, but again the suggested association was the reverse of that in the parents. There was not any evidence of association of  $R_3$  with the other pollen colour locus cream  $P_a$ , or with petalody  $Pdy$ , or with the corolla colour factor  $Y_a$ .  $R_3$  has not been adequately tested against the *anoma-*



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*lum* corolla colour factor  $Y_c$ , since the latter has not yet been established in *arboreum* in conjunction with either of the recessive anthocyanin types, ghost or spotless. Some little information on the point has been summarized in the table, derived from two small families segregating 15 : 1, and three small families segregating 7 : 1 for  $R_2$  and  $R_3$ , all segregating 1 : 1 for  $Y_c$ . Although the evidence of complete independence is not conclusive, it is obvious that  $Y_c$  is not closely associated with either of the anthocyanin duplicates. The segregation of  $R_3$  in conjunction with yellow corolla grades has been shown in Table 9 earlier. Most of the range in yellow in this cross was associated with the segregation of  $Ydp$ , and from the table it might appear that  $R_3$  and  $Ydp$  are linked, since in the ghost class yellow grading was consistently higher than in the spot class, and there was far less clear distinction into the two expected yellow classes. This has already been discussed. Furthermore, delimitation of the depressor genotypes in such a family by simple inspection was impossible, on account of the interspecific modifier segregation which was also present. From examination of similar data from other families it has been concluded that, when due allowance is made for the interaction between gold expression and corolla grade, there is no evidence of linkage between  $R_3$  and  $Ydp$ , and it has been found easy to separate them into different backcross lines.

### (8) *Summary*

(i) Red petal spot in *anomalum* is not due to a single allele as in the cultivated Asiatic species, but is the result of the complementary interaction between a ghost allele in the Asiatic  $R_2$  locus, and a spotless allele in a duplicate anthocyanin locus. This spotless gene is characterized by a pleiotropic gold-petal expression. The symbol  $R_3^{GO}$  is allocated to it.

(ii) *G. arboreum* and *G. herbaceum* carry in the duplicate locus a basal recessive allele, lacking both basic anthocyanin expression and the spot characteristic. It is symbolized as  $r_3^{OO}$ .

(iii) The *anomalum* duplicate  $R_3^{GO}$  was established in the homozygous condition in the cultivated Asiatic genotype, both as a spotless type in conjunction with  $R_2^{AO}$  and as a red-spotted type similar to *anomalum* in conjunction with  $R_2^{OS}$ . The gold appearance of the petals of these types is something quite new in Asiatic cottons.

(iv) The gold-petal expression of  $R_3^{GO}$  is very variable, being especially dependent upon yellow corolla constitution. Only when on a homozygous yellow background is gold developed to an appreciable extent.  $R_3^{GO}$  is not completely dominant, since gold is intensified in the homozygote.

In conjunction with *Ydp* on a homozygous yellow background, gold is intensified to pink. Homozygosity for  $R_3^{GO}$  does not lead to any appreciable intensification in pink beyond the expression of the heterozygote. Heterozygosity in the  $Y_a$  locus for either  $Y_a^P$  or  $y_a$  is a very potent factor in limiting the expression to a very faint tinge of gold, even when  $R_3^{GO}$  is duplex, and not even *Ydp* is able to intensify it. Heterozygosity in the  $Y_b$  and  $Y_c$  loci does not reduce gold expression. On the recessive pale petal colours and on white, gold is not expressed, except occasionally as a faint pink edge to the petal. In addition other factors such as exposure to sunlight, and other modifiers less easily analysable than the main corolla colour factors, also produce profound effects on gold and pink expression. Strains of the cultivated species, in which the gold-producing allele does not occur, differ considerably in their content of these modifiers. In *anomalum* itself, which is very pale cream, almost white, there is only a faint suggestion of pink on the petal. This is a result of the absence of the main gene for yellow flavone development essential to the full expression of the gold spotless allele, and to the fact that this species also lacks modifiers which intensify gold and pink.

(v) Part of the size of the *anomalum* spot is a subsidiary effect of  $R_3^{GO}$ . The more important component of its size constitution is a modifier of considerable magnitude fairly closely linked to the ghost gene. There was no conclusive evidence of the linkage of spot size modifiers with the  $L$ ,  $P_a$ , or  $Ne$  loci.

(vi) As a result of the low level of the *anomalum* spot size genotype, apart from these large-size components, it was possible to develop, from hybrid progeny, lines so low in their content of size modifiers that the expression of the main spot gene was reduced to extinction.

(vii) Dominance of spot modifiers is in the direction of larger size.

(viii) Using the large spot modifier associated with  $R_2^{OS}$ , it was shown that spot size modifiers are not specific to any particular allele, but affect all types of spot—ghost, full red spot, and red-spot compounds, both those due to complementary alleles at the same locus and those due to the complementaries in duplicate loci.

(ix) There was no evidence of linkage of  $R_3$  with any one of the three linkage groups  $L-Lc_1$ ,  $H_a-Lc_2$ , and  $Ne-P_b$ , or with the independent genes  $P_a$ ,  $Y_a$  and *Ydp*. Evidence of complete independence from  $Y_c$  was not conclusive, but the genes are certainly not closely associated.

## V. POLLEN COLOUR

In the cultivated Asiatic species pollen is almost invariably deep yellow in colour and there is remarkably little variability in shade. Only two exceptions have been observed. Ramanathan & Balasubramanyan (1933*b*) reported a single occurrence of "cream pollen" in a strain of *G. obtusifolium* Roxb. = *G. arboreum* var. *neglectum* forma *indica*, Hutchinson & Ghose. They found that it differed from full yellow in a single recessive gene, and there was no evidence of modifier segregation even in an interspecific cross involving *G. herbaceum*. Through the courtesy of Mr V. Ramanathan seed of the "cream pollen" strain was sent to this Station in 1934. The writer has grown it under the type number N 25, and has confirmed the above findings. In no cross studied was there any difficulty in classifying segregating progenies. Whilst on a visit to the U.S.S.R. in 1933 Dr S. C. Harland collected seed from a plant of *G. herbaceum* with cream pollen in Uzbekistan, district Tashkent. On his return he handed this material over to the writer for study. The strain, grown under the number H 16, has bred true to a pollen colour grade similar to that which characterizes the cream pollen Uplands and those wild species of *Gossypium* which have cream pollen. This grade is very much whiter than that of Ramanathan's *arboreum* "cream" N 25, and it is proposed to refer to the latter as *pale yellow*, whilst the *herbaceum* H 16 type will be called *cream*. In appearance the common full yellow, pale yellow, and cream pollens correspond very closely to the phenotypes of full yellow, pale and white corolla in the  $Y_a$  series—i.e. at grades 8, 3-2 and 1 respectively on Hutchinson's (1931) corolla colour plate. The genes responsible for the recessive cream and pale pollen colours are not however allelomorphic like the  $Y_a$  series of corolla colours with which they have been compared phenotypically. The  $F_1$  between N 25 and H 16 has full yellow pollen, and the  $F_2$  gave the dihybrid 9 : 3 : 4 ratio, in which all three terms, representing full yellow, pale and cream could be satisfactorily delimited. Data will be published shortly. The symbol  $p_a$  is proposed for the cream pollen gene, and  $p_b$  for pale pollen. On this basis the H 16 cream type is regarded as  $p_a P_b$ , and the N 25 pale as  $P_a p_b$ .

Although a series of pollen colour grades was not found necessary in studying crosses within the cultivated Asiatic cottons, they were used to some extent in studying the *anomalum* hybrids. In such cases flowers were collected in the field in the early morning and graded at about 10 a.m. in the laboratory, a necessary precaution on account of deepening

of the colour of the pollen during the day. The same scale as Harland (1929*b*) had used in his pollen colour study in New World cottons was employed. The coloured plate in his 1929 paper is not a good reproduction of his grades, but fortunately his standard strains were available. Grade 0 is white, grade 1 pale yellow, and grade 4 the deepest yellow. Practically all the yellow pollen Asiatics grade between 2 and 3, N 25 pale at 1.2, and H 16 cream at 0.3.

*G. anomalum* has cream pollen, grading at 0.1, definitely whiter than that of H 16. An  $F_1$  between them, of eleven plants, also had cream pollen, of an intermediate shade grading at 0.2. When *anomalum* was crossed with full yellow pollen types from *arboresum* or *herbaceum*, the  $F_1$  was brought down in grade to an intermediate shade of yellow. This was in strong contrast to crosses between H 16 cream and *arboresum* full yellows, which were all as intense yellow as the dominant parent. Some representative examples were as follows:

Type no.	Yellow grade	Pollen colour grade of hybrid with	
		<i>G. anomalum</i> (cream)	<i>G. herbaceum</i> H 16 (cream)
A 8	2.2-2.5	1.8-2.0	2.0-3.0
A 16	2.4-2.6	1.9-2.5	—
H 10	2.5	1.0	—
O 1	2.5	1.1-1.6	—
O 8	3.0	1.3	—

There was considerable breakdown in dominance in the *anomalum* hybrids, and this was more marked in crosses involving the *herbaceum* types H 10, O1, O8, than in those into which the *arboresum* types A 8 and A 16 entered. A backcross of *an.*  $\times$  A 8 to T 3 (a synthesized multiple recessive cream pollen segregate selected from an  $F_2$  of *arboresum* yellow pollen  $\times$  *herbaceum* H 16) gave yellows ranging from about grade 3 down to intermediate yellow of about grade 1, and a more uniform group of creams ranging slightly round 0.3. Unfortunately there was no opportunity at the time to grade each plant individually, but in spite of the variation within each group it can be said that the two main classes were quite distinct from one another. When contrasted against the creams even the low grade yellows were distinct. This backcross gave 68 yellows and 70 creams, indicating that the *anomalum* cream pollen gene is allelomorphic and probably identical with that in H 16,  $p_a$ .

The pale pollen type N 25 was also crossed with *anomalum* and with the  $F_1$  of (*an.*  $\times$  H 16 cream). The first progeny consisted of 11 plants all low grade yellow, about 1.5-2, definitely yellow when contrasted against N 25 pale itself. The second progeny of 19 plants ranged from definite full

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yellow down to light yellow, but the lightest was somewhat deeper than pale itself. This complementary reaction shows that *anomalum* carries the normal allelomorph of pale.

### VI. NECTARIES

Extra-floral nectaries are common in cotton, but there is very little published information on their inheritance, largely because of the extremely tedious nature of the observations and the great variability in expression of the character. They occur on the undersides of the leaves, on one or more of the main veins, and at the base of the bracts. If inside the bracts they occur just between them, at the outer base of the calyx tube. If outside the bracts, they occur opposite and immediately below them. On some types nectaries are completely absent from both leaves and bracts. All types of *G. arboreum* which have leaf nectaries have internal bract nectaries also; those types without leaf nectaries have no bract nectaries either. All types of *G. herbaceum* which have been observed at this Station have nectaries on the leaves, and most have internal bract nectaries as well. A few strains of var. *frutescens* lack bract nectaries, even though they have leaf nectaries. External bract nectaries, although common in New World cottons, are extremely rare in the cultivated Asiatics, having only been described from a single type of *herbaceum* from Malta (Watt, 1926).

Leake (1911) studied the inheritance of leaf nectaries in a cross between two *arboreum* types, and demonstrated that presence and absence was controlled by a single pair of allelomorphs. Subsequent work at this Station, which will shortly be published, has confirmed that within *arboreum* presence of leaf nectaries (*Ne*) is almost dominant over their absence (*ne*), but it has been found that in interspecific crosses involving *herbaceum* this dominance may break down completely. The same main gene controls leaf nectaries in both these species, and is also responsible for the formation of internal bract nectaries in *arboreum*. Since it has been found that in interspecific crosses the expression of the main gene may be limited by modifier action to the leaves only, it is highly probable that those occasional types of *herbaceum* which have leaf nectaries but no bract nectaries also carry the common main nectary gene, together with a particular genotypic complex which does not permit its full expression.

*G. anomalum* is characterized by the presence of leaf nectaries. In hybrids involving the *arboreum* no-nectary types N 5, N 14, and N 25, leaf nectaries were as distinct as in the wild species. In a backcross of (*an.* × N 14) to N 14 leaf nectaries were somewhat indistinct on some

plants, but with careful observation it was possible to classify with confidence, and a clear single-factor segregation of 81 plants with leaf nectaries, and 86 without, was obtained. Evidently the minor gene constitution of *anomalum*, so far as leaf nectaries are concerned, is very similar to that of *arboreum*, and not at all like that of *herbaceum*, which leads to dominance breakdown in crosses with *arboreum*. That the same main locus is concerned as in these species was shown by the backcross of (*an.*  $\times$  *arb.* A 8 *Ne*) to the *ne* types N 14 and T 3, which gave a total of 301 plants all with leaf nectaries.

The situation with regard to bract nectaries is much more distinctive. There are no internal bract nectaries in *G. anomalum*, as is usual in types with leaf nectaries in the cultivated Asiatics, but external bract nectaries, which are extremely rare in the latter, are present. In hybrids with the *arboreum* no-nectary types N 5, N 14, and N 25, no external bract nectaries were present except on occasional flowers, on which they were indistinct. Apart from the difference in position, this situation is more like that in *arb. ne*  $\times$  *herb. Ne* than in *arb. ne*  $\times$  *arb. Ne* hybrids. This indicates that the minor bract nectary genotype of *anomalum* is nearer to that of *herbaceum* than *arboreum*, though of course it is quite distinctive in that *anomalum* only shows externals and not internals. On analogy with the situation in the cultivated Asiatics, it seems likely that these external bract nectaries are due to the same *Ne* allele as controls the presence of leaf nectaries, together with a particular specific genotype. On the other hand, in the absence of further data, it cannot definitely be concluded that the identical allele is concerned.

## VII. FUZZ AND LINT CHARACTERS

In cultivated Asiatic cottons the hairs on the seed are differentiated into two distinct coats, one consisting of long fairly easily detached lint hairs, and the other of short strongly adherent fuzz hairs. In a few so-called "tufted" types the latter coat is represented only by a few hairs at the chalazal end of the seed. Hutchinson (1935) found tufted to be due to a partially dominant gene T, whose expression was considerably influenced by modifiers. He made use of a series of grades for classification of segregating progeny, in which grade 1 was the most highly tufted type (naked) and grade 6 almost fully covered except for small naked areas. Fully covered segregates were classified as "fuzzy". Homozygous tufted types grade 1-4, and heterozygotes usually 3-6. The symbol *Fz* has been assigned to this gene in Hutchinson & Silow's (1939) revised list of gene symbols.

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In *G. anomalum* the hairs on the seed are not separated into two distinct layers, but all appear to form a single coat of short hairs up to 10 mm. in length. These are very firmly adherent to the testa, so that when pulled off many break along the length of the hairs instead of at or very near to the point of attachment to the testa, as in commercial cottons. Nevertheless *anomalum* carries the genetic basis for fuzz, since fully fuzzy segregates have appeared in progeny derived from *an.*  $\times$  tufted hybrids. In the  $F_1$  between *anomalum* and *arboreum* A 16 tufted (grade 2-4) the hairs were also very adherent, so that when pulled they broke along their length. It was therefore not possible to obtain any estimate of the dominance of  $Fz$  in this hybrid. In the first backcross to A 16 approximately 60% of plants set seed, and on them normal ginning was possible. The following segregation was observed:

Tufted grades					Fuzzy
2	3	4	5	6	
.	.	1	15	29	64

A comparable (*arboreum* fuzzy A 15  $\times$  A 16) first backcross to A 16, gave:

Tufted grades					Fuzzy
2	3	4	5	6	
9	16	8	.	.	.

In this intra-*arboreum* cross, dominance of  $Fz$  was almost complete, and the entire segregation was at a very different level from that in the inter-specific cross. No direct comparison with the level in *herbaceum* is available, but information on the point may be gathered from the following  $F_2$  progenies, one of the same intra-*arboreum* as cited above, and the other of *arboreum* A 16  $\times$  *herbaceum* H 10 fuzzy:

Cross	$F_1$ Tufted grade	$F_2$ , percentage class frequencies							Actual no. of $F_2$ plants scored	
		Tufted grades						Fuzzy		
		2	3	4	5	6	6+	Total		
A 15 $\times$ A 16	3	.	17	34	14	14	.	21	100	29
H 10 $\times$ A 16	6+	.	7	12	13	16	6	46	100	162

In the intra-*arboreum*  $F_2$  the expected 25% of recessive fuzzy segregates was recovered, but in the *arboreum-herbaceum* cross 46% of the segregates were graded as fuzzy. From the high grading of the  $F_1$  plant, the excess of fuzzy segregates may be attributed to the inclusion of a number of heterozygotes in this class. The general displacement of the segregation was in the same direction as in the *anomalum* hybrid progeny, but to a less extreme degree than in the latter, where even the homozygous tufted class was fairly extensively covered. A selfed progeny derived from an  $F_1$

(P 2386) between H 10 and a selection from the second backcross of (*an.*  $\times$  A 16) to A 16 gave the following:

F <sub>2</sub> , percentage class frequencies							Actual no. of F <sub>2</sub> plants scored
Tufted grades					Fuzzy	Total	
2	3	4	5	6			
.	.	8	9	21	62	100	135

By a second backcross it would be expected that a certain proportion of the *anomalum* genotype might be lost, yet the displacement was still more extreme than in the H 10  $\times$  A 16 F<sub>2</sub>. That we are justified in considering this a modifier displacement and not the result of the introduction of a fuzzy allele dominant over tufted *Fz* is shown by the fact that a particular (*an.*  $\times$  A 16) third backcross to A 16 selection (15,094) when selfed, gave:

Tufted grades					Fuzzy
2	3	4	5	6	
.	.	19	27	8	7

In this progeny the fuzzy segregates were clearly recessive. That an allele in the *Fz* locus is concerned is confirmed by the following evidence. A selection from the second backcross of (*an.*  $\times$  A 16) to A 16 was selfed, giving:

Tufted grades			Fuzzy
5	6	Intermediate	
2	3	1	6

The plant classified as "intermediate" (15,245) had seeds uniformly covered with an extremely thin fuzz layer. Its subsequent behaviour showed that it was a heterozygote; heterozygotes within *arboreum* are usually patchy, with completely naked areas of variable size. Heterozygotes of this uniformly thinly covered type have been observed in *arboreum*  $\times$  *herbaceum* crosses. The plant 15,245 was crossed with N 5 and N 14, two *arboreum* fuzzy types, and two plants from each F<sub>1</sub> were selfed. Two families segregated for *Fz*, whilst two others were homozygous fuzzy. One of the fuzzy sibs of 15,245 was also crossed with N 5 and N 14, and four F<sub>1</sub> plants selfed gave over 300 progeny all of which were fuzzy. In these non-segregating families *Fz* from A 16 was clearly absent, and its place must have been taken by its allele *fz* from *anomalum*. The results cited previously show that this is not by any means the only fuzz gene of importance in this species. *G. anomalum* has an intensely fuzzy genotype, and whether it carries the genetic basis for lint at all merits further discussion.



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The most significant evidence bearing on this point is that, in crosses involving normal fully linted strains of cultivated cottons, segregation of lint characters is not discontinuous but appears to be similar to that which occurs within the cultivated species, where differences have been found to be due to typical quantitative genes of relatively small individual effect. The most satisfactory means of identifying such genes is to trace their association with main genes affecting more easily observable characters. This type of analysis is necessarily restricted to early generations, since in later generations it is impossible to be sure that crossing-over has not occurred. Unfortunately in early generations of this hybrid material only a few families gave a separation of main gene classes sufficiently distinct to be of use in this connexion, and in addition their general low fertility made observation of seed characters particularly difficult. There is therefore practically no information available on inheritance of ginning percentage, since for reliable estimates of this character about 100 seeds are required from each plant. All that can be said is that the amount of hairs on the seeds of  $F_1$  hybrids was intermediate between that on *anomalum* and on the cultivated parents. The quantity is expressed as the percentage weight of hairs on the total weight of seed + hairs. The firm adherence of the hairs made normal ginning impossible, so the hairs were cut off close to the base with scissors. Typical "ginning percentage" results were as follows:

<i>anomalum</i> = 8 %		
A 16	= 41 %	<i>an.</i> × A 16 = 28 %
H 10	= 26 %	<i>an.</i> × H 10 = 18 %

Rather more satisfactory information is available on the inheritance of hair length, since for the determination of this character only five seeds from each plant are required. On the  $F_1$  hybrids hair length (mean maximum halo length) was intermediate but rather nearer that of the cultivated parent, e.g.

<i>anomalum</i> = 8–10 mm.		
A 16	= 25–26 mm.	<i>an.</i> × A 16 = 20 mm.
H 10	= 27–29 mm.	<i>an.</i> × H 10 = 23 mm.

Even in the first backcross the mean hair length very closely approached that of the cultivated parent. Data are summarized, as mean "lint" length for contrasting pairs of main gene classes, in Table 13. In each case the main gene derived from *anomalum* is listed after that from the cultivated species.

The first three families, all first backcrosses from the same  $F_1$  *anomalum* × *arboreum* hybrid, show the association of lint length with leaf

shape segregation. In the first family, a backcross to *herbaceum* H 10, the *anomalum* leaf shape class had lint significantly shorter, by about  $\frac{1}{2}$  mm., than the laciniated class. There was no significant difference in lint length between the leaf shape classes in the other two families, which were respectively backcrosses of the same  $F_1$  to *arboreum* N 14, and to T 3, a multiple recessive selection derived from an *arboreum*  $\times$  *herbaceum*  $F_2$ , but predominantly of *arboreum* genotype. The second group of three families shows the association with  $R_2$ . These families are derived from two sib-plants from the first backcross of (*an.*  $\times$  A 8) to H 10. In each of the progenies derived by backcrossing again to H 10, the class carrying

Table 13. *Lint length association with main gene segregation*

Family	Class	n	Mean lint length mm.	d	t	P
(an. $\times$ A 8) $\times$ H 10	$L^L$	209	27.59	-0.49	2.16	0.05-0.02
	$L^A$	167	27.10			
(an. $\times$ A 8) $\times$ N 14	$L^L$	61	23.27	+0.05	0.10	0.9
	$L^A$	37	23.32			
(an. $\times$ A 8) $\times$ T 3	$L^L$	36	23.66	+0.11	0.16	0.9
	$L^A$	27	23.77			
11,757 $\times$ H 10	$R_2^{AO}$	70	27.37	-1.41	4.32	Very small
	$R_2^{OS}$	61	25.96			
11,703 $\times$ H 10	$R_2^{AO}$	22	28.68	-1.76	2.21	0.05-0.02
	$R_2^{OS}$	12	26.92			
11,703 $\times$ T 3	$R_2^{AO}$	79	28.13	+0.26	0.52	0.6
	$R_2^{OS}$	41	28.39			
(an. $\times$ A 16) $\times$ A 16	$r_3^{OO}$	42	25.23	-1.02	2.19	0.05-0.02
	$R_3^{GO}$	64	24.21			
(an. $\times$ N 14) $\times$ N 14	$r_3^{OO}$	18	24.22	-0.84	0.77	0.5
	$R_3^{GO}$	18	23.38			
(an. $\times$ N 14) $\times$ N 5	$r_3^{OO}$	16	25.12	-0.69	0.84	0.4
	$R_3^{GO}$	23	24.43			

$R_2^{OS}$  from *anomalum* had lint about  $1\frac{1}{2}$  mm. shorter than that of the spotless class, and this difference was significant. In the third family, a backcross of one of the sibs to T 3, there was no significant difference in lint length between the two genotypes. There is thus evidence of lint length genes on both the  $R_2$  and  $L$  chromosomes, and an indication, from the differences in the backcrosses to *arboreum* and *herbaceum*, that the lint length alleles carried by these two species on these chromosomes are not the same. A similar difference between these two species in spot size genes on the  $L$  chromosome has already been mentioned. The third group of families, showing association of lint length with  $R_3$ , were all backcrosses of (*anomalum*  $\times$  *arboreum*) to *arboreum*. In the first of these the class carrying the *anomalum* gold allele had lint about 1 mm. shorter

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than the non-gold class. This difference was significant. In the other two families the lint length differences were of much the same magnitude, but in the small numbers available did not reach the level of significance. Lint length genes are evidently located on the  $R_3$  chromosome also. Other small families, not indicated in the table, gave no evidence of association of lint length with pollen colour and leaf nectary segregations, but could not be considered conclusive in this respect. The lint length effects associated with the  $L$ ,  $R_2$  and  $R_3$  genes from *anomalum* were of just the same degree of magnitude as Hutchinson (1932*b*, 1934) found to be associated with the first two of these loci within the cultivated species. Exactly the same type of gene appears to be responsible for the difference between *anomalum* and the cultivated cottons as is responsible for differences within the cultivated species themselves. Their lint was not acquired at a single step, but was probably built up gradually, either by the development of a morphologically new structure, or by a process of differentiation in a single coat of hairs such as occurs on the seed of *anomalum*. In that case such types as *anomalum* are not likely to be of any value as a source of lint length or ginning out-turn genes. In view of the strength and fineness of its seed hairs, however, it might well be worth paying attention to this species as a source of lint fineness.

#### VIII. LINT COLOUR

Very little information on the inheritance of lint colour in Asiatic cottons has been published. In view of the fact that most of the few studies which have been reported were interspecific (Kottur, 1923; Ramanathan & Balasubramanyan, 1933*a*), it is not surprising that they have yielded no clear conception of the situation. Experience at this Station has indicated that some of the main lint colour genes are extremely susceptible to modifier action, and that there are great differences between strains in their modifier content. There are two classes of main genes. In one class are those whose expression is a dark brown or khaki, and whose potency is of such magnitude that they are almost fully dominant even in interspecific crosses. In the other class are the light brown genes whose expression is very dependent upon the minor genotype; their presence in certain very near-white cottons would not be suspected, and it has only been possible to confirm it by the intensification which occurs in certain crosses as a result of the introduction of modifiers, and with the help of a close linkage with the lintless  $h_a$  gene which facilitates analysis enormously. Without this assistance it would not have been possible

to arrive at any definite conclusions with reference to gene homologies within the light brown class.

Within *arboreum* two independent khakis are known. One of these Hutchinson (1934) has located on the leaf shape chromosome, at a distance of 30 units from the latter gene. For this khaki gene, which Hutchinson termed K, the symbol  $Lc_1^K$  is proposed. The other khaki has been found to be closely linked with  $h_a$ , and will be termed  $Lc_2^K$ . Two light brown lint genes are known; one of them, which is allelomorphous with the second khaki gene, is widespread in *herbaceum* and is occasionally encountered in *arboreum*. This will be symbolized as  $Lc_2^B$ . The other light brown gene,  $Lc_3^B$ , is independent of the former two lint colour loci, and has been identified only very rarely and in association with  $Lc_2^B$  in *herbaceum*, when the homozygous combination is somewhat darker in expression than the khaki genes. It should be mentioned that on certain relatively stable modifier backgrounds, when dealing with the two light browns, an approximation to the expected duplicate gene segregation is sometimes encountered. This is especially so within *arboreum*. Hutchinson (1935) has reported such a case. In general *herbaceum* is at a much lower lint colour modifier level than *arboreum*. For the classification of segregating progeny a series of lint colour grades has been established; grade 4 is the darkest brown; the khakis grade at 2; whilst 0 is white. In modifier studies it has been necessary to interpolate observations in between the primary colour standards numbered 0, 1, 2, 3 and 4.

The lint of *G. anomalum* is a medium brown of grade 3. The lint of  $F_1$  hybrids involving white-linted *arboreums* grades at about 1.6. The most useful information is derived from backcrosses to *arboreum* white (N 14) of the hybrid *an.*  $\times$  A 8, which is a dark brown  $Lc_1^K$  strain. Data are also available from a backcross to T 3, a synthesized multiple recessive from an *arboreum*  $\times$  *herbaceum* cross, with white lint. The results show that it has a predominantly *arboreum* lint-colour genotype, so they will be considered along with those from the backcross to N 14. A wide range in lint colour segregation occurred, intergrading from dark brown to light brown, together with some whites which formed a distinct class. The families were scored as follows:

Cross	Lint colour grades								Total		
	4	3	2.6	2	1.6	1	0.5	0	Brown	White	Ratio
( <i>an.</i> $\times$ A 8) $\times$ N 14	13	25	15	18	14	15	1	16	101	16	6.3 : 1
( <i>an.</i> $\times$ A 8) $\times$ T 3	.	40	.	.	7	10	.	8	57	8	7.1 : 1

The presence of whites indicates that *anomalum* cannot carry  $Lc_1^K$ , which

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is known to be almost fully dominant. The 7:1 backcross ratios are suggestive of a three factor segregation. There is further evidence that in addition to the dark brown gene introduced by A 8, two other light brown genes were present. An attempt was made to determine if  $Lc_2^B$ , the light brown linked with lintless, were present, by crossing *anomalum* with the lintless strain N 19 and backcrossing to lintless, but none of the progeny grown set seed. A light brown of grade 1 was therefore selected from the backcross of (*an.*  $\times$  A 8) to N 14, and crossed with N 19 lintless, and a brown-linted segregate again backcrossed to a lintless strain. Ordinarily lintless segregates cannot be scored for lint colour; this is a serious disadvantage from the standpoint of linkage observation, but a strain carrying  $h_a$  was available, which, by reason of its modifier content, had some lint on its seed. This strain, T 6, has been described elsewhere under the number P 2417 (Silow, 1939b).  $h_a$  segregates in backcrosses to this strain can be recognized by the glabrousness of their stems, a pleiotropic expression of the main gene not affected by the modifiers which induce sparse lint on the otherwise lintless seed. The following segregation in this backcross was observed:

		Lint colour grades		
		1.8-1.5	1	0
$H_a$		9	7	
$h_a$		.	10	13

$Lc_1^K$  from A 8 had clearly been lost from this material, in view of the absence of segregates of grade 2. The absence of whites amongst the fully linted  $H_a$  segregates indicated the presence of the linked  $Lc_2^B$ , which must have been derived from *anomalum*. On the other hand if only this gene had been present, there should have been no browns in the  $h_a$  class, in view of the closeness of the linkage concerned. That approximately 50% of the  $h_a$  segregates were brown indicates the presence of a second brown lint gene independent of  $h_a$ . This must also have come from *anomalum*. By way of contrast, a sib selection from the backcross to N 14, similarly treated, gave:

		Lint colour grades	
		2	0
$H_a$		17	23
$h_a$		15	28

In this line the  $Lc_1^K$  gene from A 8 only was present, giving a very different segregation from the previous family in which the two light brown genes from *anomalum* were present. The evidence indicates that

no more than these two main genes are responsible for the lint colour of *anomalum*. Whether the second of them is homologous with  $Lc_2^B$  is not known. The reasonably clear demarcation between browns and whites in the backcross of the *anomalum*  $\times$  *arboreum* A 8 hybrid suggests that the minor genotype of these two species is very similar. From experience with *herbaceum*  $\times$  *arboreum* hybrids, it can be stated that there would have been far more intergrading between browns and whites if the modifier complex of *anomalum* had been at all like that of *herbaceum*.

#### IX. THE COMPLEMENTARY FACTOR LETHAL "CRUMPLED"

In certain interstrain crosses Hutchinson (1932*a*) reported the appearance of a peculiar semi-lethal or even lethal type of abnormality which he described as "crumpled". He showed that this was due to the interaction of two complementary factors which he named A and B, and which have since been assigned the symbols  $Cp_a$  and  $Cp_b$  (Hutchinson & Silow, 1939).  $Cp_a$  was found in only one strain of *arboreum* var. *soudanensis*, grown under the type number N 9, but  $Cp_b$  was present in approximately half of the 29 strains of the several varieties and forms of *arboreum* and *herbaceum* which he tested. There were absolutely no phenotypic indications of the presence of  $Cp_a$  or  $Cp_b$ , but when strains carrying these genes were crossed the resultant progeny exhibited the crumpled characteristic, its expression being dependent upon the modifier background. In the least extreme case, involving a strain of *arboreum* forma *bengalensis*, the plants were stunted in growth, with shortened internodes, abnormal development of vegetative buds giving a witch-broom appearance, and irregular crumpled brittle leaves, and they set only a very few small bolls. In the majority of crosses involving other *arboreum* types the seedlings were much more abnormal and completely sterile, developing little if at all beyond the cotyledon stage, and the cotyledons themselves in most cases were very thickened and crumpled. This may be regarded as the typical expression of crumpled within the *arboreum* complex, but an even more extreme expression occurred in crosses involving five of the six *herbaceum* strains which carried  $Cp_b$ ; in crosses between these and the  $Cp_a$  strain only empty seeds resulted. Such extreme behaviour only occurred in the case of one Chinese strain amongst the eleven *arboreum* types found to carry  $Cp_b$ , and the formation of empty seeds is to be regarded as a typical expression of this complementary lethal in crosses involving *herbaceum*.

Since 1932 a large number of other crosses has been performed at this

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Station, giving further evidence on the distribution of these genes.  $Cp_a$  has been found again in a second accession of material similar to N 9 from the same general locality in the Anglo-Egyptian Sudan, in the Blue Nile district bordering on Abyssinia, but has not been encountered elsewhere. Altogether 128 different strains representing all the geographic subdivisions of both *arboreum* and *herbaceum* have been crossed with types known to carry the  $Cp_b$  gene, and all have given normal progeny.  $Cp_b$  has been found in 25 of 41 representative strains tested; both phases of this gene have been found in all the geographic groups which have been investigated, except the Indo-African *herbaceum* varieties *frutescens* and *africanum*, all five tested strains of which have been found to carry  $Cp_b$ . Only the *arboreum indica* and *soudanensis* groups have not yet been adequately examined.

*G. anomalum* has been crossed with several strains carrying  $Cp_b$ , such as A 8, N 19, and O1, and with all of these has given normal progeny. The wild species evidently does not carry  $Cp_a$ . The  $Cp_a$  strain N 9 was crossed with *anomalum*, and all 43 seedlings which were obtained had crumpled cotyledons and died before the exsertion of any plumular leaves. In appearance these crumpled seedlings were similar to those which are characteristic of crosses within *arboreum*. The  $F_1$  (*an.*  $\times$  A 8) was crossed with N 9; 74 seeds were obtained on the  $F_1$  as female, and 46 on N 9 as female; 49% of the former and 37% of the latter were empty or contained imperfectly developed embryos, ranging from very minute about one-twentieth normal size to nearly full size. Examination of some 1500 seeds derived by backcrossing the same hybrid to ten other *arboreum* and *herbaceum* types which did not carry  $Cp_a$  also showed from 15 to 50% of empty or imperfectly developed seeds. Thus although Hutchinson showed that in crosses involving *herbaceum* the development of empty seeds was due to the complementary lethal mechanism, their occurrence in the cross of (*an.*  $\times$  A 8) to N 9 can only be considered an expression of general hybrid incompatibility. From the fully developed seeds 46 seedlings were established, and all were crumpled, almost exactly as in the direct cross of *anomalum* to N 9, except that three of them exserted from 1 to 5 minute rudimentary leaves before dying. That no normal seedlings appeared indicates that the crumpling factor carried by *anomalum* is at the same locus as in *arboreum*, and it is evident that these two species are very similar in their minor crumpling genotype.

## X. LEAF SHAPE

It has already been shown (Silow, 1939a) that the leaf shape of *anomalum* is controlled by a member of the *arboreum-herbaceum* leaf shape multiple allelomorph series. This gene,  $L^4$ , which has not been recorded from the cultivated Asiatic species, is accompanied in *anomalum* by a lobe-broadening genotype similar to that which distinguishes *herbaceum* from *arboreum*.

## XI. DISCUSSION

(1) *Genetic aspects; flower pigmentation interactions*

The genetic information and the observational records of interaction now available suggest that the genus *Gossypium* would form very suitable material for a biochemical study of the developmental relationships between anthoxanthins and anthocyanins, which would be the more interesting in that some of these pigments show very striking specific distributions. Within *arboreum* all three dominants  $Y_a$ ,  $Y_b$ , and  $Y_c$  are required for the production of full yellow corolla. From such flowers Perkin (1916) was able to isolate the glucosides gossypitrin and isoquercitrin. In the same species  $y_a$  leads to the development of white flowers with petals some 25% shorter than yellows. From these Perkin isolated only a small quantity of a substance resembling apigenin in its general properties, a flavone known to occur in several other ivory-white flowered plants. Like these, the white flower of *arboreum* gives the typical flavone reaction on fuming with ammonia. The intermediate pale yellow  $Y_a^P$  at the same locus is much nearer white than full yellow in its low intensity of colour, but much nearer yellow than white in its very slight reductive effect on length. At each of the other two loci only one recessive allele is known, and both are phenotypically pales. They can be distinguished from one another with reasonable confidence when on a common stable background. In view of the possibility that lower alleles at these loci may eventually be found, like white at the  $Y_a$  locus, these pales have also been designated as  $Y^P$ . The Chinese pale  $Y_b^P$  is much like the Burmese pale  $Y_a^P$ , being very slightly paler in shade than the latter, and only very slightly shorter than full yellow.  $Y_c^P$ , which is confined to and characteristic of *anomalum*, is definitely slightly yellower than the other two pales, yet shows much the same degree of shortening of the petal as does white. Corolla size appears to be physiologically associated with the main colour classes, since no recombinations which could be attributed to crossing-over between linked genes have ever been



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observed. The indication, from the small number of observations available, that the double recessive  $Y_b^P Y_c^P$  is intermediate in petal length rather than as short as  $Y_c^P$ , if substantiated, would imply that the mechanism by which shortening of the petal is effected is not the same in all cases. It seems very likely that the three pale corolla types differ chemically, and full yellow and white certainly do, so that an investigation of these substances might throw interesting light on the relation of certain organic radicals to growth processes.

Various strains and species of cotton are characterized by a red corolla. As judged by differences in their appearance and in their reaction with the flavones, at least two different types of anthocyanins are concerned. As an example of one of these types, the *anomalum*  $R_3$  gold-petal allele may be taken. It has been shown (Table 11) that its expression is very dependent upon yellow corolla constitution, changes in genotype at the  $Y_a$  and  $Y_{dp}$  loci especially being associated with variations in intensity of anthocyanin on the petal lobe. In the absence of the gold allele  $Y_a$  is almost though not fully dominant as far as yellow intensity is concerned, but heterozygosity for pale or white at this locus lowers the expression of  $R_3^{GO}$  very considerably. This is interpreted as indicating a correlation between production of anthocyanin and yellow flavone, such as Lawrence & Scott-Moncrieff (1935) demonstrated in *Dahlia*. Thus although  $Y_a$  appears to be of high phenotypic dominance, it is actually of low dominance as far as quantitative production of pigment is concerned. Hutchinson (1931) has shown that this gene is not fully dominant in the petal length effect either. Heterozygosity for pale in the  $Y_b$  or  $Y_c$  loci was not found to affect gold expression, suggesting either that the physiological dominance relationships at these loci is different from that at the  $Y_a$  locus, or that these loci control the development of different types of yellow or pale flavone. In the presence of duplex recessives at any one of the three  $Y$  loci gold hardly attains expression at all. Interaction with the yellow depressor,  $Y_{dp}$ , which is restricted to *anomalum* in its natural distribution, is particularly instructive. Since this gene diminishes the intensity of pales as well as of full yellow, it seems likely that it restricts the quantity rather than that it affects the type of flavone, i.e. it acts as a general and not a specific anthoxanthin suppressor. When on a homozygous yellow background the yellow depressor intensifies the expression of  $R_3^{GO}$  from gold to pink. That this is a real and not an apparent intensification due to the lowering of intensity of the yellow background is supported by the fact that  $Y_{dp}$  does not intensify gold on heterozygous yellow to the slightest extent,

though the yellow background is depressed. This incidentally affords further evidence of the physiologically cumulative effect of  $Y_a$  dosage. The antagonistic interaction between anthocyanin and flavone which is indicated by the concurrent suppression of yellow and intensification of gold to pink, gives strong support to the theory of Lawrence & Scott-Moncrieff (1935) of a limited pigment source common in part to both anthoxanthins and anthocyanins. That this is not the whole of the story, however, is evident from the fact that, in the absence of the depressor, cyanic intensity is positively associated with  $Y_a$  dosage. Lawrence & Scott-Moncrieff described a yellow inhibitor in *Dahlia* which appears to be similar to  $Ydp$ , and which also increases cyanic intensity. In view of their opinion that the production of specific pigments is simultaneous rather than successive, they suggested that the yellow inhibitor reduced the productive and competitive power of the yellow flavone gene at the source. They found that the cyanic intensification was due to a reduction in the proportion of pelargonin to cyanin. Whether the intensification in the case of  $R_3^{GO}$  is likewise qualitative, or whether it is quantitative in nature is not known, and in view of the complications which they observed it would be futile at this stage to attempt an interpretation of the mode of action of the several genes concerned in *Gossypium*. It does however seem clear that  $Ydp$  must act after the inception of the pigment source. The fact that the depressor inhibits yellow flavone production in both simplex and duplex  $Y_a$ , but that this results in cyanic intensification in the latter genotype only suggests that a threshold level is involved and that at least some pigment production reactions of this type are not necessarily simultaneous but may be sequential. Confirmatory support of this hypothesis is available from a totally different source. The typical cultivated Asiatic cotton flower has a yellow corolla, and a red spot due to the gene  $R_2^{AS}$ . In the fresh flower anthocyanin is restricted to the red spot at the base of the petal, the petal lobe being totally devoid of any red pigment. As the flower wilts towards the end of the first day of anthesis the petal lobe acquires a faint cyanic tinge, and by the following morning the anthocyanin intensity is very much as in the *anomalum* type of "medium to intense" gold (Pl. 18, figs. 9, 10). The petal lobe of Chinese pale-yellow corolla plants carrying the spot allele is also devoid of anthocyanin when fresh, but as the flowers age they acquire a much more intense coloration than do full-yellow spotted plants, going even beyond the intensity of the "pink" shown in Pl. 18, fig. 12. Here also then there are indications of antagonistic interaction between flavone and anthocyanin production, and in this case there can be no doubt that the pro-

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duction of anthocyanin is subsequent to that of flavone. The situation suggests that, as far as their control of anthocyanin production in the petal lobe is concerned, the distinction between the gold petal gene, which initiates anthocyanin production early in the life of the flower, and the ordinary spot gene, which induces anthocyanin formation in the petal lobe only as the flower fades, lies entirely in their reaction rates.

As an example of the second of the anthocyanin types in *Gossypium* the so-called "*sanguineum*" type, which occurs in *arboreum*, may be taken. This has an intense wine-red petal lobe, very different from gold both in appearance and in its interactions, which have been described by Hutchinson (1932*b*). Perhaps the most striking difference is that variability in its expression is almost entirely in terms of distribution on the petal, and to a much lesser extent in intensity, as may be seen from Hutchinson (1932*b*, Pl. XXVI). His grades 1-11 represent increasing degrees of extension of red from the margin to cover the entire surface of the petal. Most  $Y_a Y_a$  types with red grade at 11,  $Y_a^P Y_a^P$  types at 2-4, and  $y_a y_a$  types at 1-2. Heterozygosity of yellow for pale or white in the  $Y_a$  locus has only a slight effect in restricting expression of red to grade 10, whereas gold under the same conditions is reduced from intense-medium to faint or very faint. There is not a great deal of information on the effect of heterozygosity in the  $Y_b$  or  $Y_c$  loci, but in some families heterozygous both for  $y_a$  and  $Y_b^P$  or  $Y_c^P$  there was no degradation below that involved in the case of  $Y_a y_a$  alone. In a family heterozygous for  $Y_a y_a$  and for the depressor however reds ranged only from 7 to 9, i.e. expression of red was reduced in the same general direction as when yellow grade is lowered by main gene constitution. Unfortunately no data are available for this red on duplex  $Y_a$  with  $Y_{dp}$ . Taking into account the evidence available on interaction with flavone genotype, and the very different colour of the *sanguineum*  $R_2^{RS}$  red as opposed to the  $R_3^{GO}$  gold, it appears very likely that these two anthocyanin types differ qualitatively. From phenotypic appearance it is probable that the New World tetraploid *hirsutum*  $R_1$  red petal is similar in nature to the *arboreum*  $R_2^{RS}$ . When this varies in expression it also does so chiefly in distribution. However in its most extreme variants it has never been observed to cover anything like so great a proportion of the petal as does the  $R_2$  red either in *arboreum* or when transferred to *hirsutum* (Harland, 1935). In this case then, with respect to their petal red effects as distinct from their spot effects<sup>1</sup>, it appears plausible that the *hirsutum* and *sanguineum* reds represent only *quantitative* variants of the same basic type.

<sup>1</sup> The *arboreum* red is spotted, whilst the *hirsutum* red is spotless.

The diploid American *aridum* has a pink petal, and from work in progress it has been established that the allele responsible is homologous with  $R_1$  of the tetraploid species. It has been observed that the petal colour of many of the segregants derived from backcrosses of *aridum* to the tetraploid species is very suggestive of *anomalum* gold, and as in the latter, variation is primarily in intensity rather than in distribution on the petal. It must be remembered that there is no genetic evidence that  $R_1$  and  $R_3$  are not homologous, and that the latter symbol subscript was adopted because it is unlikely that they are of "recent" homology in view of Skovsted's theory that the New World tetraploids are allopolyploids derived from an Asiatic and an American diploid species or cytologically similar types. Quite apart from this, if the assumption is correct that the *hirsutum* and "gold" reds differ qualitatively, the demonstration that *aridum* red, similar to gold, is located at the *hirsutum*  $R_1$  locus implies that qualitative variants may occur at a single anthocyanin locus. This is quite plausible in view of the fact that alleles which control the production of full yellow and pale yellow flavone, which are almost certainly different, are known to occur at the same locus along with one which determines ivory flavone. Such a qualitative anthocyanin series has been described in *Callistemma* by Wit (1937), who attributed the formation of pelargonidin, cyanidin and delphinidin derivatives to different members of a triple allelomorph series. On the other hand Lawrence & Scott-Moncrieff attributed the formation of specific anthocyanins, not to individual genes, but to a general balance between all anthocyanin and anthoxanthin factorial contributions. It is interesting to note that they consider this to be an uncommon situation resulting from the competition of homologous factors in an allo-octoploid. It is highly probable that such a derivative complication may occur in *Gossypium* also, superposed upon the possibility of specific factorial control. Skovsted (1933) considers that in this genus even the so-called diploid species with  $n=13$  are secondary polyploids. He based this opinion on the occurrence of secondary pairing, which by itself is not necessarily a valid indication of homology (Heilborn, 1936). Support is however given to his interpretation by the occurrence in the diploid species of genetic replication, some types of which of late years have come to be regarded as evidence of cytological replication also. Thus in *arboresum-herbaceum* there is triplication of lint colour loci (this paper, § VIII):

$Lc_1^K$	$Lc_2^K$	.	(Khaki)
	$Lc_2^B$	$Lc_3^B$	(Light brown)
$lc_1$	$lc_2$	$lc_3$	(White)

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Genetic duplication for anthocyanin has also been demonstrated in *anomalum*. This is an unusual type and its interpretation as such will be further substantiated below. It is probable that in cotton certain complementaries also may be regarded as replicated loci. Though argument on these lines could very easily be carried too far, it is to some extent justified in *Gossypium* by the parallelism in mutant steps which is exhibited in some of these loci, such as those affecting corolla colour and pollen colour:

Corolla colour	$Y_a^aP$	$Y_b^bP$	$Y_c^cP$	(Yellow)
	$Y_a^a$	$Y_b^b$	$Y_c^c$	(Pale)
	$y_a$	.	.	(White)
Pollen colour	$P_a$	$P_b$	.	(Yellow)
	.	$p_b$	.	(Pale)
	$p_a$	.	.	(Cream)

If these cases of complementaries be regarded as replication, then there are two cases of triplication. Skovsted (1937) has suggested that the basic 13 in this genus represents five chromosomes duplicated and only one triplicated, so that at first sight it appears that there are too many cases of genetic replication to fit in with the suggestion that they indicate cytological replication, there being no evidence of linkage between any of the lint colour and corolla colour loci. Yet the suggestion cannot be turned down on these grounds alone at this stage of our knowledge. In other genera various cytological mechanisms have been involved in speciation, and some of these, such as replication of parts of chromosomes, may also have played their part in evolution within *Gossypium*. Winge (1938) has extended the underlying idea to its logical conclusion in his hypothesis of the taxonomic importance of polymery, pointing out the stabilizing influence, in units of higher taxonomic rank, of the frequent replication of particular factors throughout the genom as a result of polyploidy, duplication and translocation.

The interpretation of the *anomalum* anthocyanin locus as a duplicate of that in *arboreum* and *herbaceum* requires special explanation. The most common anthocyanin expression in the latter, red-tinged stem and red-petal spot, is due to a single allele in the  $R_2$  series. Two lower members of this allelomorphous series are known, though they are relatively uncommon. They are respectively ghost spot, which lacks the capacity to produce red colouring matter, and spotless, which is similar to red petal spot in vegetative expression. In compound these two lower alleles resemble the common red petal spot phenotype. Of course this compound type never breeds true. *G. anomalum* also has a slightly red-tinged stem and red

petal spot, similar in size and appearance to that typical of most of the cultivated Asiatic cottons, but genetically it is constructed in a totally different way. It is not, as in the latter, due to a single allele, but is the result of the complementary interaction between a ghost gene in the Asiatic  $R_2$  locus, and a spotless gene in another locus,  $R_3$ . This type of compound spot is naturally true breeding. Although these particular  $R_2$  and  $R_3$  genes act as complementaries, the loci are regarded as duplicates for two reasons:

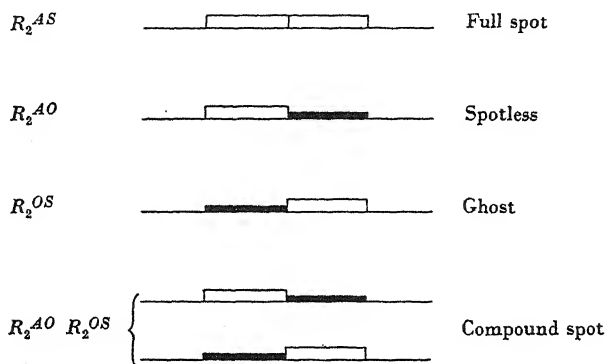
(i) The  $R_3$  allele is comparable in type and behaviour with known members of the  $R_2$  series in *arboreum-herbaceum*.

(ii) If spotless were not known in the latter series, it would not have been possible to identify the presence of a spotless gene in the  $R_3$  locus, and the *anomalum* spot would have behaved as if it were due to an identical duplicate of the Asiatic full spot allele.

Just as in the cultivated species other members of the  $R_2$  series are known which have intense red pigmentation of either petal lobe, plant body, leaves, or calyx, as pleiotropic effects of the main anthocyanin spotted or spotless allele, so the *anomalum*  $R_3$  spotless gene is characterized by a gold-petal lobe. Here again, as in the leaf shape series of allelomorphs which have recently been discussed (Silow, 1939*a*) it looks as if there are at least two independently variable systems within the anthocyanin series, one involving presence and absence of spot, the other presence and distribution of anthocyanin. Thus in *arboreum* red-leaf types and red-tinged stem types, both with and without spot, are known. Hutchinson (1934) in discussing the organization of the gene, envisaged the anthocyanin locus as embodying a protosome with two gene centres on which a series of episomes are attached, with one centre controlling presence or absence of basal anthocyanin, and the second centre determining distribution of anthocyanin in the plant. If this basic concept be extended to include the further information on anthocyanin inheritance in cotton now available, the two gene centres may be better conceived as controlling respectively presence and distribution of anthocyanin, and presence and absence of petal spot. The arrangement of the  $R_2$  series of alleles in *arboreum*, as listed at the beginning of § IV of this paper, conforms to this scheme. It seems highly probable that spotless equivalents of the other spotted alleles  $R_2^{RS}$ ,  $R_2^{OS}$  and  $R_2^{OO}$  may be found in the future. It is likely that the spotless equivalent of the latter,  $r^{OO}$ , exists in the  $R_3$  locus in *arboreum* (see § IV (2)). This scheme takes into account

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the complementary nature of ghost and spotless types in giving the spot phenotype, e.g.



This conception may also be extended to the  $R_3$  locus. It is very likely that, with the discovery of further allelomorphs in the future, it may be found that the anthocyanin possibilities in the petal lobe form a third system varying independently of vegetative anthocyanin and petal spot.

It is tempting to speculate on the possibility that New World spots are not in fact true full spots in the Asiatic sense, but perhaps ghosts segregating on a uniformly spotless background. All New World types without red spot are spotless. The ghost phenotype is not known. It is of interest in this connexion that the progeny of Upland spotless (*hirsutum*)  $\times$  tetraploid N 14 ghost<sup>1</sup> (*arboreum*) show red spot. At present there is no indication as to the function of red petal spot in the economy of the plant, though that it may be of some significance is suggested both by its very frequent presence not only in *Gossypium* but in several related genera as well, and by the complicated and unusual genetic mechanism by which it is attained in *anomalum*. Yet from some of Wright's (1931) mathematical treatments of the problems involved in variability and differentiation in natural populations it is clear that many specific distinctions may be without any adaptive value whatsoever.

#### (2) *Speciation in the genus Gossypium*

This study of inheritance in interspecific crosses has clearly demonstrated the genetic processes involved when the plant breeder makes wide crosses in order to "break the type", completely new characters being built up by the combination of genes which do not normally occur together in nature. Similar studies in several other plants and animals

<sup>1</sup> Doubled by colchicine treatment by Mr S. G. Stephens of this Station.

have led to certain deductions as to the mechanisms by which species become differentiated. These have recently been reviewed by Harland (1936), who pointed out that in all cases the process of gene substitution was involved, and he illustrated this from the results of his own experiments in New World cottons. The genus *Gossypium* is particularly favourable material for this type of investigation in that it incorporates a number of well-defined species, several of which yield highly fertile hybrids. Indeed this situation in early days led to considerable difficulty in interpretation of genetic results in this genus, since contrasting pairs of characters were frequently only available in different species. Harland's realization that this involved segregation in many minor genes or modifiers as well as in the main ones under study quickly cleared up the situation, and he has had considerable success in interpreting results following "transference" to a uniform background stabilized by recurrent backcrossing. The application of this method has made possible the genetic analysis of many characters in cotton, and on the basis of the extensive type collection available at this Station it has been possible to formulate a reasonably comprehensive picture of the make-up of several species. However, in this genus, as in others, genetic comparisons have so far been limited to a restricted portion of the wide range of specific differentiation exhibited. Thus Harland's information on differences amongst the New World species, and that of Hutchinson in the Old World cottons, has referred to groups of species so closely related amongst themselves that their hybrids are fully fertile in the first generation, and only show some degree of breakdown in the second and subsequent generations—obviously the minimal degree of distinction which can be regarded as of specific status. The first generation hybrids of *anomalum* and the cultivated Asiatic cottons are only 1% fertile on selfing, and 10% fertile on backcrossing, so that the examination may now be extended to cover a much wider range of specific divergence—the widest in fact which can be subjected to analysis before complete sterility imposes an insurmountable obstacle to genetic study. Admittedly, differences in fertility by themselves must be accepted with caution as indications of relative magnitude of specific differentiation, but in this case, that the distinction involved between *anomalum* and the two cultivated Asiatic species is much greater than that between the latter themselves is supported by morphological differences which have led some taxonomists to consider the wild African species as even generically distinct.

Several evolutionary processes have been at work in *Gossypium*. Skovsted (1934) has shown that the diploid species may be separated



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broadly into an Asiatic and an American complex, between which there is cytologically practically no homology. The American tetraploids appear to be allopolyploids containing both the Asiatic and American diploid complements. Interspecific hybridization followed by amphidiploidy has therefore played an important part in the evolutionary history of *Gossypium*, but this Lotsyan type of evolution has undoubtedly been both preceded and followed by the more gradual evolutionary process of gene substitution. The most significant information bearing on this process is likely to be derived from a study of the situation within any one of the basic groups of species, though in view of Skovsted's (1937) hypothesis that even the diploids are secondary polyploids, it will be impossible to be sure which of the differences found are the result of gene substitution and which the result of polyphyletic.

The lowest grade of speciation which it is possible to recognize as such, that in which the first appearance of sterility and breakdown in viability is delayed to the second hybrid generation, is well exemplified by the two cultivated Asiatic diploid species, *G. arboreum* and *herbaceum*. Their differences in genetic structure with reference to the main and minor gene constitution of eight different characters have been assembled in Table 14 from Hutchinson's publications and subsequent work by the author, some of which is in course of publication. Side by side with this information that from *anomalum* is also tabulated for comparison. It is important to point out that in the *anomalum* crosses every character was investigated which was at all amenable to analysis, particularly with reference to the genes so far known in the cultivated cottons, so that the results presented in Table 14 are as complete as is reasonably possible. The only characters on which adequate observations were not made were on such quantitative variations as in intensity of hairiness, and breadth and lacination of bracts, in which it was felt that the only outcome of laborious observations was likely to be an addition to the already lengthy series of vague statements that "the character was controlled by multiple factors". In Table 14 the symbol + indicates the genotype of the one and only accession of *anomalum* which has been available for study. Herbarium collections do not indicate any great variability within this species. In the two cultivated species, of which a type collection of some 200 lines is available, together with much herbarium and published information, there is considerable variability. In these the relative frequency of occurrence of different alleles is indicated. In estimating the modifier status of a species the terms high and low have been used in a strictly conventional sense, being termed "high" if the modifiers are

such as to enhance the expression of the character in the same direction as the dominance of the main gene. Thus in the case of the leaf shape series where dominance is in the direction of narrower lobed phenotypes, the modifier background of *arboreum* is such that the expression of a particular main gene is narrower than in *herbaceum*, and so is considered relatively "high".

From Table 14 it will be seen that *arboreum* and *herbaceum* are characterized by the same main gene in thirteen of the fifteen loci tabulated. The only tendency to divergence in these loci lies in the strikingly different frequencies with which certain of the less common alleles occur in the two species, as in the  $Y_a$ ,  $Y_b$ ,  $R_2$ ,  $P_a$ ,  $P_b$ ,  $Lc_1$  and  $Lc_3$  series. Some of the rarer of these alleles have been recorded in only one or other of the two species. In the  $L$  series *herbaceum* is characterized by an allele which is also very common in *arboreum*. In the latter species other alleles, one of which is equally common, occur in addition; these have not been recorded at all in *herbaceum*. In  $Lc_2$ , the remaining one of the fifteen loci, is the only case where there does appear to be a fairly constant difference between these two species, though even here the distinction is by no means absolute.  $Lc_2^B$  is the most frequent allele at this locus in *herbaceum*, but  $lc_2$  is sometimes present; and although the latter is characteristic of *arboreum*, the higher member does occasionally occur. Thus we may summarize that there is not any striking difference between the two cultivated Asiatic species as far as their main gene constitution is concerned. Practically all of the difference lies in the modifier systems, which are quite distinctive though usually nothing like so different as in the case of *anomalum*. Nevertheless in at least seven of the eight characters studied there is wider modifier segregation in inter-specific than in most interstrain crosses, or the species may even be characterized by an obvious displacement in expression of the main allele, as in the case of leaf shape and lint colour.

Much greater divergence in genetic constitution is apparent in *anomalum*. In this species, in only nine of the fifteen loci examined are the same genes present as are common in or typical of the cultivated species. There is no point in trying to make out of this a proportionate estimate of similarity. The number of genes in which *anomalum* is similar to the other two species must be very high, since this figure must include all those genes which might be described as familial or generic, as opposed to specific. There can be no doubt that much the same normal alleles or ones so similar as to be almost indistinguishable must occur in *anomalum*, as in *arboreum* and *herbaceum*, at the loci which occasionally carry the

Table 14. A comparison of the genetic constitution of three diploid species of *Gossypium*

Character	Constitution	arborescens	herbaceum	anomalum
Corolla colour	$Y_a$ yellow $Y_a$ pale	<b>Typical form</b> Restricted to Assam, Bengal and Burma, where it is fairly frequent	<b>Typical form</b> Very rare; only once recorded in var. <i>frutescens</i>	+
	$y_a$ white	Restricted to northern India, Burma and China, where it is fairly frequent	Not recorded	
	$Y_b$ yellow $Y_b$ pale	<b>Typical form</b> Restricted to Burma and China, rare	<b>Prevailing form</b> Not recorded	+
	$Y_c$ yellow $Y_c$ pale	<b>Prevailing form</b> Not recorded	<b>Prevailing form</b> Not recorded	+
	Yellow intensity modifier status	—	Somewhat below that of <i>arb.</i> , especially in var. <i>typicum</i> . Much nearer <i>arb.</i> than <i>an.</i>	<b>Distinctive</b> ; in aggregate low, primarily on account of the specific member <i>Ydp</i>
Anthocyanin	$R_2^{HS}$ full red	Occurs in Africa, northern India, Burma and Java; uncommon	Not recorded	
	$R_2^{LS}$ red leaf	Restricted to China, where it is uncommon	Very rare; only once recorded, in <i>typicum</i>	
	$R_2^{CS}$ red calyx	Not recorded	Rare, in <i>typicum</i> only	
	$R_2^{AS}$ red spot	<b>Typical form</b> Restricted to Burma and China, where it is uncommon	<b>Typical form</b> Not recorded	+
	$R_2^{OS}$ ghost	China, where it is uncommon	Not recorded	
	$R_2^{LO}$ red spotless	Very rare; only once recorded, from China	Not recorded	
	$R_2^{AO}$ spotless	Very rare; only one or two records from China	Very rare, in <i>typicum</i> only	
	$R_3^{GO}$ gold spotless $r_3^{OO}$ spotless	Not recorded	Not recorded	+
	Spot size modifier status	<b>Prevailing form</b> —	<b>Prevailing form</b> Somewhat below that of <i>arb.</i> , especially in var. <i>typicum</i> . Much nearer <i>arb.</i> than <i>an.</i>	<b>Very distinctive</b> ; in aggregate the level is much as in <i>arb.</i> and <i>herb.</i> ; since there is one very potent large size gene on the $R_2$ chromosome, the balance of the modifier system must be at a relatively low level
Pollen colour	$P_a$ yellow $p_a$ cream	<b>Prevailing form</b> Not recorded	<b>Typical form</b> Very rare; only once recorded, in <i>typicum</i>	+
	$P_b$ yellow $p_b$ pale	<b>Typical form</b> Very rare; only once recorded, in <i>indica</i>	<b>Prevailing form</b> Not recorded	+
	Yellow intensity modifier status	—	Variable; some strains similar to <i>arb.</i> , others slightly lower	<b>Distinctive</b>

Nectaries	<i>Nc</i> present <i>ne</i> absent	Typical form Common	Prevailing form Not recorded	+
	Leaf nectary modifier status	—	Distinct from that of <i>arb.</i> and <i>an.</i>	Intermediate, but very near <i>arb.</i>
	Bract nectary modifier status	—	Distinct from that of <i>arb.</i> , but much nearer <i>arb.</i> than <i>an.</i>	Very distinctive in that external bract nectaries are present
Seed fuzz	<i>Fz</i> tufted	Uncommon except in China	Very rare; not known if the occasional tufted types which occur are really due to this gene	
	<i>fz</i> fuzzy	Typical form	Typical form	+
	Fuzz intensity modifier status	—	Lower than that of <i>arb.</i>	Much lower than in <i>herb.</i>
Lint colour	<i>Lc<sub>1</sub><sup>K</sup></i> khaki <i>lc<sub>1</sub></i> white	Infrequent Typical form	Not recorded Prevailing form	+
	<i>Lc<sub>2</sub><sup>K</sup></i> khaki <i>Lc<sub>2</sub><sup>B</sup></i> light brown <i>lc<sub>2</sub></i> white	Infrequent Typical form	Not recorded Typical form Probably not common	+
	<i>Lc<sub>3</sub><sup>B</sup></i> light brown <i>lc<sub>3</sub></i>	Not recorded Prevailing form	Occasional Typical form	+
	Brown intensifier status	High	Low	As in <i>arb.</i>
Crumpled lethal	<i>Op<sub>a</sub></i>	Very rare; only once recorded	Not recorded	
	<i>cp<sub>a</sub></i>	Typical form	Typical form	+
	<i>cp<sub>b</sub></i>	Common	Common	+
	<i>cp<sub>b</sub></i>	Common	Common	
	Modifiers	Crumpled usually lethal in cotyledon stage	Empty seeds	As in <i>arb.</i>
Leaf shape	<i>L<sup>L</sup></i>	Very rare, only once recorded	Not recorded	
	<i>L<sup>N</sup></i>	Fairly frequent, though probably confined to <i>cernuum</i>	Not recorded	
	<i>L</i>	Common, especially in Bengal	Not recorded	
	<i>L<sup>A</sup></i>	Not recorded	Not recorded	+
	<i>l</i>	Common, especially in China and southern India	Prevailing form	
	Leaf narrowing modifiers	High	Low	Low as in <i>herb.</i>

rare and frequently deleterious recessive mutants such as the five types of lintless, chlorophyll deficiency, petalody, curly leaf, etc. It is the relatively few main loci, six in number, in which these species differ, which are of significance. *G. anomalum* is characterized by an allele at  $R_2$  which is uncommon in *arboreum* and unknown in *herbaceum*, by a brown lint gene which is uncommon in *herbaceum* and unknown in *arboreum*, by a cream pollen gene at  $P_a$  which in spite of much search for this type of gene has only once been encountered in *herbaceum* and not at all in *arboreum*, and by alleles which are quite unknown outside this species at the  $Y_c$ ,  $R_3$ , and  $L$  loci. In modifier constitution *anomalum* is also strikingly distinct from the two cultivated species, the hybrid progenies showing very much wider segregation than do crosses between the latter species. In modifiers of corolla colour, anthocyanin intensity, spot size, pollen colour, bract nectaries, and seed fuzziness the wild species is very distinct from both of the cultivated ones; in leaf shape modifiers it is much nearer *herbaceum* than *arboreum*, and in leaf nectaries, lint colour, and crumpled there was no evidence that it differed appreciably from *arboreum*, though in modifiers of all four latter characters the two cultivated species are markedly distinct from one another. In Table 15 an attempt has been made to give a diagrammatic representation of the extent to which *anomalum* is considered to differ in modifier constitution from the other two species, by the horizontal displacement of the symbol  $\times$  from the two dotted lines which are intended to give a conventionalized picture of the relative modifier level in the cultivated species.

In this table the modifier constitution is depicted in relation to each of the characters studied, and not in relation to each of the independent genes controlling a particular character. This is justified by the fact that the interaction between genes is not direct but by way of their somatic manifestations (Silow, 1939*a*) so that in general it is unlikely that a set of modifiers will be restricted in their action to particular genes. The demonstration (§ IV (6) of this paper) that the spot size modifiers extracted from *anomalum* affect all types of spot, including ghost, full red spot, and those due to complementary alleles either at the same or at duplicate loci, affords an excellent example of the general non-specificity of modifiers as far as the alleles and loci affecting any particular character are concerned.

The final stage in differentiation is seen in those species which are so distinct that their hybrids, if obtainable at all, are completely sterile. In *Gossypium* such sterility is not confined to crosses between species

with different chromosome complements, but may also occur between species within any one cytological grouping. Although the geneticist cannot do a great deal with such material, the differences are in many cases so distinct that there can be no doubt that many of the main genes as well as their modifiers are dissimilar.

We thus arrive at the generalization that the more widely species within this genus are separated, as estimated by the level of fertility of their hybrids and by the number of morphological characters by which they are distinguished, the greater the magnitude and the number of the

Table 15. *The modifier situation—a diagrammatic representation of the extent to which anomalum (indicated by ×) differs from arboreum and herbaceum in modifier constitution*

Character	arboreum	herbaceum	
Corolla colour			×
Anthocyanin			×
Pollen colour			×
Leaf nectaries	×		
Bract nectaries			×
Seed fuzz			×
Lint colour	×		
Crumpled	×		
Leaf shape			×

genes in which they differ. It is quite impossible to give any absolute estimate of the magnitude of specific differences in terms of genes, but on the assumption that there is only one modifier affecting practically every main gene, it may be hazarded that the minimum degree of speciation recognizable as such is associated with a difference in at least 50% of the loci involved—bearing in mind in this connexion that there is also a necessarily unknown proportion of the genotype which must be

common to all species in the genus. Actually the number of minor gene differences is probably somewhat greater than this rather conservative estimate, though perhaps not much greater. There is a general impression that modifiers are very numerous and of almost infinitesimally small effect, but it does not seem that the situation is necessarily quite so extreme as this. It has been striking that in favourable material in which it has been possible to identify individual modifiers, four at least have quite considerable potency, although of course not comparable with that of the main gene. It may well be asked whether those particular modifiers have only been identified on account of the magnitude of their effect, but this is definitely not the case, since three of the four were identified almost entirely on account of particularly favourable genetic circumstances. Thus the spot size modifier carried by *anomalum* was identified on account of its linkage with the ghost allele, and by itself is sufficient to account for all of the upward extension in range in spot size in the hybrid progenies. The two lintless modifiers (Silow, 1939*b*) were identified because they affected only one of the two pleiotropic effects of the main lintless gene, so that it has been possible, in a wide segregation, accurately to delimit the main gene phases, a very necessary proviso in the analysis of any interaction system. These two genes were of surprisingly high potency, and in recent work it has been possible to follow them through with considerable confidence. They alone are sufficient to account for practically all of the overlap between two main classes which in narrow crosses are absolutely distinct from each other. The fourth large modifier, yellow depressor *Ydp*, was the only one identified solely on account of the magnitude of its effect, after separation from other interfering factors by backcrossing. This gene was sufficient by itself to account for practically all the downward extension of corolla colour in the interspecific crosses. Although two of these four modifiers were isolated from wide crosses between species which, as has already been shown, tend to differ in genes of relatively large effect, two of them, the lintless modifiers, were found in different varieties of one species. It is evident that a very limited number of such modifiers, with slight variability in expression, would simulate a typical "multiple factor" segregation, and be sufficient to give the apparently complete continuity which characterizes interspecific segregations.

The genic situation in the two cultivated New World allotetraploid species *hirsutum* and *barbadense* is somewhat intermediate between that in the cultivated Asiatics on the one hand and in *anomalum* on the other, although on the basis of morphological criteria of taxonomic importance

and the high fertility of their hybrids they would appear to be hardly more distinct than the two cultivated Asiatics. Harland (1936), in a review of his work in the New World cottons, has tabulated the main gene constitution of *barbadense* and *hirsutum*, and this may be summarized on the following lines. The genes controlling fuzz characters will be ignored, as their relationships in the two species are not fully understood. The S series and the  $R^B$  series should not be cited separately, as they have been found to be allelomorphic (Harland, 1932). Contorta has since been found to be allelomorphic with crinkled (Hutchinson, unpublished data). This leaves some 13 main loci; in the revised terminology of Hutchinson & Silow (1939)<sup>1</sup> they are  $R_1$ ,  $R_2$ ,  $Y_1$ ,  $Y_2$ ,  $P$ ,  $Lc_1$ ,  $Lc_2$ ,  $Lg$ ,  $Cr$ ,  $L$ ,  $V$ ,  $Chl_1$ , and  $Chl_2$ . At seven of these loci the two species are characterized by the same main allele; in most of these the less common alleles are quite rare and have usually been recorded in only one or other of the species. In four other loci ( $Y_1$ ,  $P$ ,  $Chl_1$ ,  $Chl_2$ ) the two species are characterized by different alleles, but these are not confined entirely to the species in which they are most common. In the remaining two loci ( $R_2$  and  $L$ ), both of which contain multiple series, each of the two species is characterized by alleles which are either absent from or extremely rare in the other species. Thus in their main loci these two species show a slightly greater degree of differentiation than the two cultivated Asiatic cottons. Of course their amphidiploid nature itself gives greater scope for divergence, there being no proof that they are monophyletic in origin. Modifier segregation also appears to be somewhat wider in crosses between *hirsutum* and *barbadense* than in those between *arboresum* and *herbaceum*, in that it is usually more difficult to separate the main gene phases; in some cases, as in that of certain members of the spot series, this may be due to the lesser distinction between the alleles themselves, but it is improbable that this is the general situation. On the other hand, it must be remembered that the New World cultivated cottons, being tetraploids, have a greater potentiality for variability than the diploids, and in the absence of a common basis of comparison it cannot be said that the apparently greater modifier segregation necessarily indicates a wider differentiation.

It must not be presumed that each of the species of *Gossypium* is characterized by a uniform modifier constitution. Within each of the four widely-distributed cultivated species there is very considerable

<sup>1</sup> In this scheme genes in the Asiatic cultivated species are indicated by symbols in italics; those in New World cultivated cottons by symbols in bold-faced type, or in italics if proven homologous with loci in the Asiatic species.



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diversification in both main and minor genes, so that the geographic forms and many local strains have come to be characterized by markedly distinct complexes with regard to particular characters. Considerable information on this phase of subspecific divergence is available from the two cultivated Asiatic species. Hutchinson & Ghose (1937 *a*) have recently revised the taxonomy of these species, and the trends within them may best be discussed on the basis of their classification. Within *arboreum* they established three varieties, two of which were based on the distinction between the annual and perennial habit. Each of these two varieties, the perennial *typicum*s and the annual *neglectum*s, were separated into the same four geographic forms. Actually from a purely genetic viewpoint it would have been preferable to give the more fundamental geographic trends the higher varietal status; the acquisition of the annual habit is a recent tendency superposed upon these, occurring independently in most parts of the distribution area of the species. As, however, the geographic trends are not associated with any distinctive tags of value to the morphologist, Hutchinson & Ghose did not feel justified in giving them varietal status and relegated them to the subordinate position. Of the four geographic forms, probably the southern Indian *indica* includes the largest proportion of primitive types.—Practically all members of this group have broad leaves (*l*) and yellow flowers ( $Y_a$ ). They have medium long and moderately fine lint, but are low ginners (Hutchinson & Govande, 1938). This group is characterized by the presence of modifiers of lintlessness, which are members of the genotype controlling the density of lint hairs on the seed; elsewhere in *arboreum* these particular genes only occur in China (Silow, 1939 *b*). The African *soudanensis* group is very similar but slightly more variable in simple morphological characters like leaf shape and anthocyanin, narrow leaf (*L*) and the rare full red ( $R_2^{RS}$ ) occurring occasionally. The central, northern and eastern Indian *bengalensis* types tend to be more hairy than other *arboreum*s; narrow leaf is more common here than elsewhere, almost completely supplanting broad in some areas; in some localities full red is fairly common, and both pale ( $Y_a^P$ ) and white ( $y_a$ ) flowers as well as the more common yellow occur. Lint tends to be shorter in this group than in any other, and as a result of its coarseness these types are heavy ginners. Lintless modifiers have not been found amongst them. The fourth geographic section in the classification of Hutchinson & Ghose, *burmanica*, is not by any means a homogeneous natural grouping in that the Burmese cottons appear to be quite distinct from those in China. The Burmese types are a variable lot. Pale and white flowers, and ghost spot

occur. Narrow leaf, rare in China, is common in some parts; and brown lint is far more frequent than in China. Some of the Burmese cottons are fairly long and reasonably fine, in contradistinction to the *bengalensis* types. As in the latter group, however, the lintless modifiers are absent. Further, there is a well-marked tendency for the Burmese group to develop rather large bracts. Burma and Manipur are parts of or a direct extension of the natural area of distribution of *arboreum*, but cottons are not endemic in China, having been introduced from various distinct sources and, in keeping with this fact, they are a somewhat heterogeneous assemblage. From historical records it appears that the two primary routes of importation were overland from Bengal-Assam to the Yellow river basin (presumably *bengalensis* types) and by sea from Indo-China to the Yangtse valley (presumably *burmanica* types, Hutchinson, 1938). The majority of present-day *bengalensis* and Burmese *burmanica* strains appear to lack the lintless epistatics, yet types carrying them are common in both the Yellow river and Yangtse basins. This may indicate that some of the Chinese cottons have originated from sources other than those so far suggested, such as the southern Indian *indica*, or the present distribution of epistatics may be the result of local selection trends from the original prototype. Red leaf ( $R_2^{LS}$ ,  $R_2^{LO}$ ) and spotless ( $R_2^{40}$ ) types, not recorded elsewhere in *arboreum*, occur in China, and ghost spot ( $R_2^{OS}$ ) is not uncommon. Though white flower occurs, the common pale does not appear to do so, but another pale type, carrying  $Y_b^P$ , is fairly common in certain localities. Tufted seeded types ( $Fz$ ) are also fairly frequent in China, but have not been recorded elsewhere except rarely in *indica*. Chinese cottons are characteristically broad-leaved, and narrow, though it occurs, is quite rare. In addition, a number of unusual forms such as curly leaf, virescent bud, yellow seedling, red margin petal, and "tinged" ghost spot, not recorded elsewhere in the Asiatic cottons, have recently been reported by Yu (1940*a*, *b*, and personal communications). China now appears to be an important secondary centre of variability in this species.

The third of the varieties of *arboreum* is a group of cottons confined to a limited tract of hill country in Assam and Bengal, characterized by high frequencies of the otherwise uncommon alleles  $Y_a^P$  and  $L^N$ , and by an unusual elongation of parts of foliar origin such as leaves, bracts, petals and bolls. This group was sufficiently distinctive in morphological characters to be given varietal status, but it appears to be only a specialized offshoot either from or closely related to forma *bengalensis*, and like the latter group has short coarse lint and a high ginning capacity. It

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will be seen that genetically the three varieties of *arboreum* are not by any means equal in ranking, the forms of the first two varieties being equivalent to the third variety in status. They are also of much the same status as the three varieties in *herbaceum*, but in this species geographic separation is associated with morphological distinction. Thus the southern African *africanum* cottons are strongly monopodial, with small thin leaves and short coarse lint. The African and western Indian *frutescens* types have larger more rugose leaves, and are intensely hairy. Amongst them occur some of the longest and finest of the Asiatic cottons. The Levant-Turkestan *typicum* group is composed of very early sympodial cottons, many of which are low in their content of yellow corolla and spot size modifiers, and they are very variable in simple morphological characters—for example, the rare alleles  $R_2^{CS}$ ,  $R_2^{LS}$  and  $R_2^{AO}$  occur here. Eastwards within this area there appears to be a strong tendency to develop a further distinctive group, with bolls which hardly open when ripe (Chernyakovskaya, 1930; Bordakov & Ivanova, 1935). In this species the three main geographic subdivisions, which Hutchinson and Ghose regarded as varieties, appear genetically to be more or less intermediate in status between the varietal and formal groups in *arboreum*. The situation within the two cultivated Asiatic species admirably illustrates the difficulties underlying efforts to harmonize morphological and biological classifications.

These examples of genetic divergence show that the differences which distinguish geographic groups within a species are of exactly the same kind as those which separate related species within a genus, though they do not attain the level at which even delayed fertility breakdown occurs. It is therefore reasonable to believe that, in some cases at least, the species themselves must have developed by an accumulation of just such small changes in emphasis in particular directions, and in geographic forms we evidently see the beginnings of the dynamic tendency which culminates in the totally different genetic structure of some homologous characters in related species. If species have developed by such an accumulation of certain tendencies, it would not be surprising to find that in some respects they had not diverged at all. In this connexion it is significant that whilst *anomalum* was found to be very distinct in genotype from both *arboreum* and *herbaceum* in many respects, in some characters it was more like one of them, whilst in others it was nearer the second species. Whether the changes in gene content are always adaptive in the Darwinian sense, as Harland (1936) implied, or whether some of them are merely fortuitous, as Wright's calculations show is possible, it is difficult to

decide. Harland has pointed out that in *barbadense* the presence of dominant main genes is frequently associated with the presence of plus modifiers, whilst in *hirsutum* the presence of the recessive allele is associated with a low modifier level. The same situation also exists to a limited extent in the Asiatic species, but is not so well marked as there is a less clear-cut allocation of a series of dominants to one species and of a series of recessives to another. One such instance is afforded by leaf shape, to which attention has been directed elsewhere (Silow, 1939a). In *arboreum*, in which narrow alleles occur, their expression is accentuated by narrowing modifiers, whilst in *herbaceum* and *anomalum*, in which only broad alleles occur, the modifier complex enhances the expression of the main gene in the direction of greater broadness. Such cases suggest that with respect to these characters a definite selection pressure has been in operation, to which the genotype as a whole, both main and minor genes, has responded. In the case of lint colour however the situation is the reverse of this. In *arboreum* light brown lint genes are rare, but this species is at a high modifier level, whilst *herbaceum*, which is characterized by main brown lint genes, has on the whole a background which minimizes their expression. *Anomalum* is characterized by brown lint genes and high modifier level. Such a situation suggests that here the fixation of the genotype may have been purely fortuitous. There are also instances, as in the case of nectaries and fuzz, where there are considerable differences in minor genotype amongst the three Asiatic species, in the absence of any main gene distinction. It is possible that the fuzz situation is to some extent a reflexion of the action of artificial selection under cultivation. Neither leaf nor bract nectaries however have any obvious significance in the economy of the plant, nor does the location of the latter either inside or outside the bracts appear to be of consequence, so that the variations in the genotype affecting these characters appear to be, following Wright's terminology, "accidents of sampling", just as in lint colour. Yet caution in applying such an interpretation is necessary. Many of the less common alleles are very localized in their occurrence in *arboreum* and *herbaceum* (Table 14). As in the case of nectaries, they do not appear to have any direct adaptive value, but the possibility that they have been selected on account of certain less obvious pleiotropic effects cannot be ignored, and the danger in assuming that such characters are neutral from the viewpoint of selective value may be illustrated from Hutchinson's (1936) survey of crop populations. In certain contiguous areas in central India he found striking differences in the frequencies of particular leaf-shape and flower-colour alleles, and these

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characters, which are known to be independent in their inheritance, showed an unexpected and strong association. The implication is that certain combinations of genes under particular circumstances have a selective advantage over other combinations, though for what reasons is not clear.

Some reference to the isolation mechanisms for the maintenance of species distinctions in *Gossypium* is necessary. Here as elsewhere there cannot be much doubt that physiological and geographic barriers are of paramount importance, but there are also other mechanisms involved. At first sight the most important of these might appear to be the complementary lethal (crumpled) system. A similar system has been described in *Crepis* by Hollingshead (1930). Some but not all strains of *C. tectorum* were found to carry a gene which acted as a lethal in hybrids with certain other species. Whether it was the whole genotype of the latter, or only a single gene as in cotton, which reacted with the *tectorum* gene, it was not possible to demonstrate. Hollingshead concluded that since the lethal was effective only in interspecific crosses, and these were almost sterile anyway, it was unlikely that the gene had played any part in the late evolutionary history of these species, though it may have been of some importance in the early stages of differentiation. In *Gossypium* the system is effective even within a single species. One of the two genes concerned is widespread though not universal in both *arboreum* and *herbaceum*; it was also found in the strain of *anomalum* tested, but whether it is prevalent in that species is not known. The other gene has however only been found in one locality in *arboreum* and not at all in *herbaceum*. It is true that that one locality is within the general distribution area of *anomalum*, but it is very improbable that the two species concerned occur in the same ecological area (see introduction). The system may have been of importance as an isolation barrier at some past time, and might easily become so again under particular local conditions, but, as in *Crepis*, it does not appear to have any great significance at the present time. The whole situation in *Gossypium* points to lack of harmony as the fundamental cause of interspecific incompatibility in this genus as it exists now, and, as has been shown, geographic separation appears to be a potent influence in the accumulation of many small differences into a clear distinction. The figures cited in § II show how even a slight change in genetic constitution can be responsible for the difference between success and complete failure in species hybridization. Further evidence on these lines is available. In discussing the difficulty in hybridizing *G. davidsonii* with other members of the American diploid

section, Skovsted (1937) suggested that the complementary lethal mechanism might be involved, and pointed out that the fertile progeny of hybrids between Asiatic cottons and *anomalum* would be satisfactory material for testing the hypothesis. He had already (1935) reported a striking difference in the relative success of *davidsonii* pollinations on *anomalum* and the cultivated Asiatic species. On *anomalum* he obtained a full set of perfectly good viable seeds; on *arboresum* and *herbaceum* he also obtained a full set, but of some 1200 seeds all were completely empty. The writer has pollinated *davidsonii* on the various derivatives of *anomalum* hybrids with *arboresum* and *herbaceum*, and the results are shown

Table 16. *The result of davidsonii pollinations on derivatives of anomalum hybrids with arboresum or herbaceum*

Plant no.	Description	Total no. of seeds	Percentage		
			Empty	Imperfectly developed	Good*
P 237	$F_1$ ( <i>an.</i> $\times$ <i>arb.</i> A 8)	266	42	52	6
14,859	( <i>an.</i> $\times$ A 8) 3rd B.C. to <i>arb.</i> A 8; only obvious <i>an.</i> gene = $Y_{dp}$	209	64	36	0
14,350	( <i>an.</i> $\times$ H 10) 2nd B.C. to <i>arb.</i> N 14 selfed; only obvious <i>an.</i> genes spot size modifiers	684	38	62	0
14,280	( <i>an.</i> $\times$ H 10) 2nd B.C. to <i>arb.</i> N 14, selfed; only obvious <i>an.</i> gene = $L^A$	589	37	53	10
P 1265	( <i>an.</i> $\times$ H 10) 2nd B.C. to <i>an.</i> ; only obvious <i>herb.</i> gene = $Y_c$	52	0	48	52
14,875	( <i>an.</i> $\times$ A 8) 3rd B.C. to <i>an.</i> ; only obvious <i>arb.</i> genes = $L^L$ and $P_a$	123	23	46	31

\* These seeds germinated and gave rise to vigorous hybrids which flowered freely. Thus by using *anomalum* as a bridging species it has been possible to secure combinations between *davidsonii* and the cultivated Asiatic cottons which cannot be obtained directly. Unfortunately all plants were completely sterile.

in Table 16. These figures, though difficult of interpretation, do not lend any support to the hypothesis that the difference between *anomalum* and the cultivated Asiatic cottons in their compatibility with *davidsonii* is dependent on any simple gene mechanism such as is concerned in the complementary lethal system, but on the contrary points rather to the importance of general genotypic balance influencing ability to hybridize.

Genotypic disharmony must also constitute an important barrier between species even after successful hybridization. Quite apart from those disharmonies so extreme as to lead to abortion of the developing embryo in the early stages, obviously unsuccessful genic combinations were frequent in *anomalum*-Asiatic hybrid progeny. Harland has also reported them within the New World section of the genus. In parts of

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India mixed crops of *arboreum* and *herbaceum* are grown, and although these species intercross freely and their first generation hybrid is vigorous and productive, Hutchinson (1938) has stated that within crop populations "hybrids of later generations are rare, and the integrity of the two species is maintained unimpaired". In the course of this report attention has frequently been directed to the deficiency of genes introduced from *anomalum* into the cultivated Asiatic species. Deficiencies were not observed with all genes, nor always with the same gene. Very few first backcrosses grown which gave clear monogenic segregation were sufficiently large to be of significance, but those which were gave on the whole fairly good segregation—e.g. leaf shape, corolla colour, pollen colour, leaf nectaries. Some of the most striking aberrations were in later generations, and amongst them a deficiency of the introduced gene was far more common than an excess. As particular examples of the deficiency of the *anomalum* gene the following may be cited:

(a) The five sib-backcrosses from (9992  $\times$  A 16) (§ III (3)). 9992 was a selfed derivative of a third backcross of *anomalum* to *arboreum*. The five backcrosses from (9992  $\times$  A 16) to A 16, using the hybrid derivatives as seed parent, were homogeneous amongst themselves, and gave 212  $Y_c$  : 149  $Y_c^P$  (from *anomalum*).

(b) Two selections from a first backcross to the cultivated species, 11,703 and 11,757, when again backcrossed (§ IV (3)), gave four homogeneous progenies with 469  $R_2^{AO}$  : 344  $R_2^{OS}$  (from *anomalum*). Here again the hybrid derivatives were used as seed parent.

(c) P 1822, a selection from a second backcross to *arboreum*, gave even more striking deviations. When backcrossed as seed parent it gave 25  $R_3^{GO}$  (from *anomalum*) : 43  $r_3^{OO}$ , and as pollen parent 10 : 20. When selfed it gave only 12 : 23 where a 3 : 1 ratio had been expected. If the deficiency is due to any disturbance in gametogenesis it is not confined to the male or female side alone. Even more striking deficiencies of the same type have been found by Hiorth (1933) in backcrosses of interspecific hybrids in *Collinsia*, and Skovsted (unpublished data) has also observed a deficiency of an anthocyanin allele in backcrosses of *G. aridum* to New World cultivated cottons. Clearly there can be under certain circumstances a severe selective elimination of foreign genes, which occurs either in gametogenesis, or, in view of the frequent poorly developed seeds found in *anomalum* derivatives, in the early zygotic stages. Apparently the disadvantage is associated with those genoms which contain the greater proportion of foreign material, again pointing to the importance of a harmonic balance within the genotype. Whatever

the mechanism involved, it must act as a potent stabilizing influence against contamination between related species.

## XII. SUMMARY

Very different degrees of specific divergence are represented within the Asiatic diploid section of the genus *Gossypium*. Hybrids between the cultivated species *arboreum* and *herbaceum* are fully fertile in the first generation, and only show breakdown in viability and fertility in the second, but hybrids between those species and the wild African *G. anomalum* are almost sterile, though fortunately not completely so. This paper deals with the inheritance in the latter hybrids of eight characters. The genetic structure of the three species is compared in terms of the fifteen main loci involved, and their associated minor genes. The same main loci are represented in all three species, but different alleles may enter into the construction of homologous characters. A particular and unusual instance is afforded by the anthocyanin petal spot common in this genus. In the cultivated Asiatic cottons its characteristics are determined by a single allele; in *anomalum* they are the result of genes, situated in duplicate loci, which act as complementaries—a conception not to be confused with that involved in the more usual interpretation of complementary factors. Genetic support is given to the contention, hitherto based on cytological grounds, that the diploid species are themselves derived polyploids. The closely related species *arboreum* and *herbaceum* differ hardly at all in their main loci, but their modifier systems are quite distinct. *Anomalum*, which is taxonomically considerably further removed from these two species than they are from one another, shows a much wider divergence in main loci, and whilst in some characters the minor genotype is near that of either one or other of the cultivated species, in others it is very distinct from both. The genetic situation in the three species seems to be a further development of the type of “incipient speciation” which is seen in the geographic differentiation occurring within the species. Some of the differences between species, where both main and minor genes are working in the same direction, appear to be adaptive. Others give no positive indication of being anything other than purely fortuitous.

## ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Mr J. B. Hutchinson for invaluable discussion and helpful criticism during the preparation of this report. This investigation was initiated under the guidance of Dr S. C.



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Harland, to whom I am also deeply indebted for his interest and advice. Thanks are also due to Mrs C. T. Watts for her kindness in painting the types reproduced in Pl. 18.

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## EXPLANATION OF PLATE 18

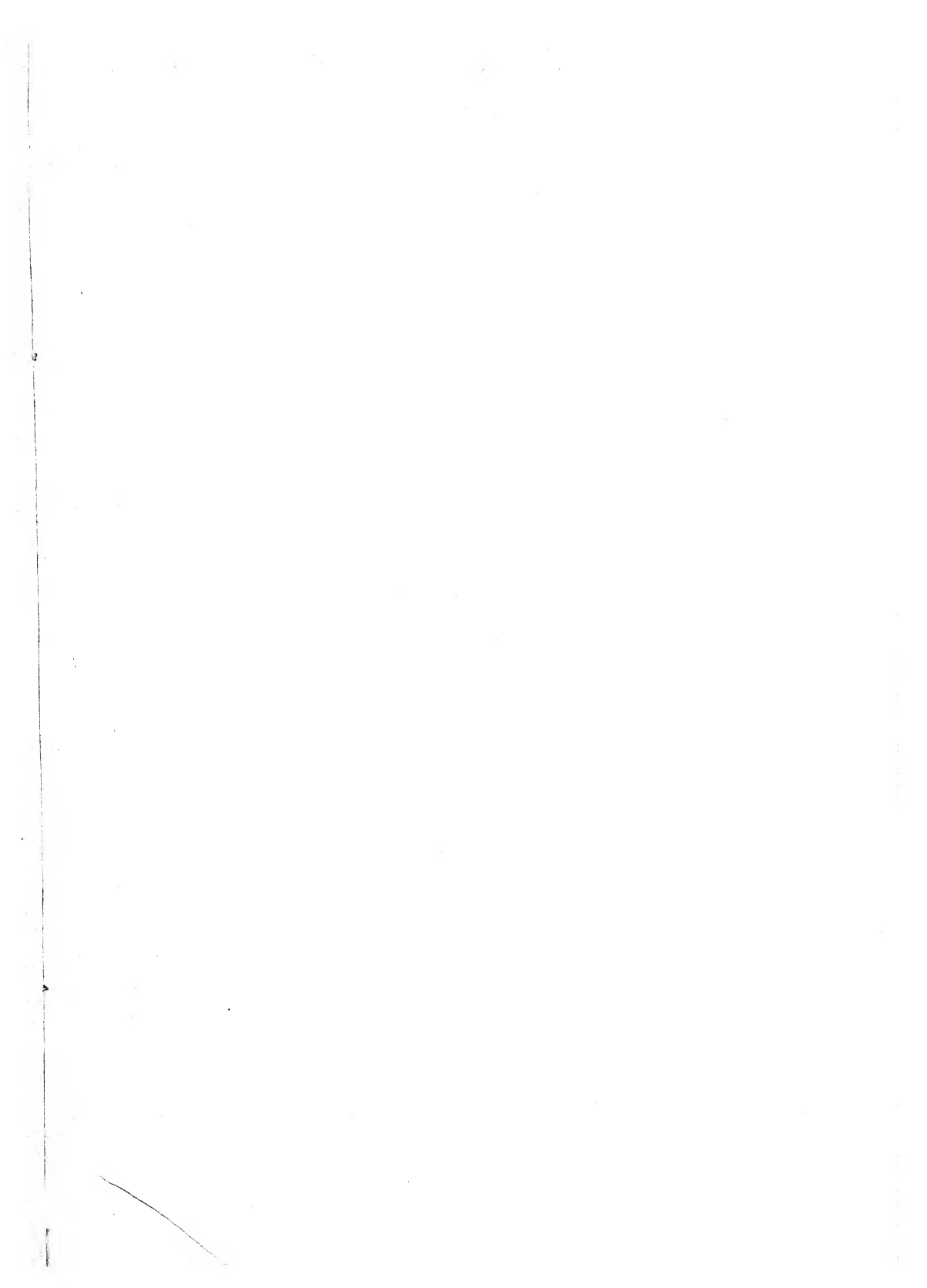
Anthocyanin and corolla colour in derivatives of *anomalum* hybrids.

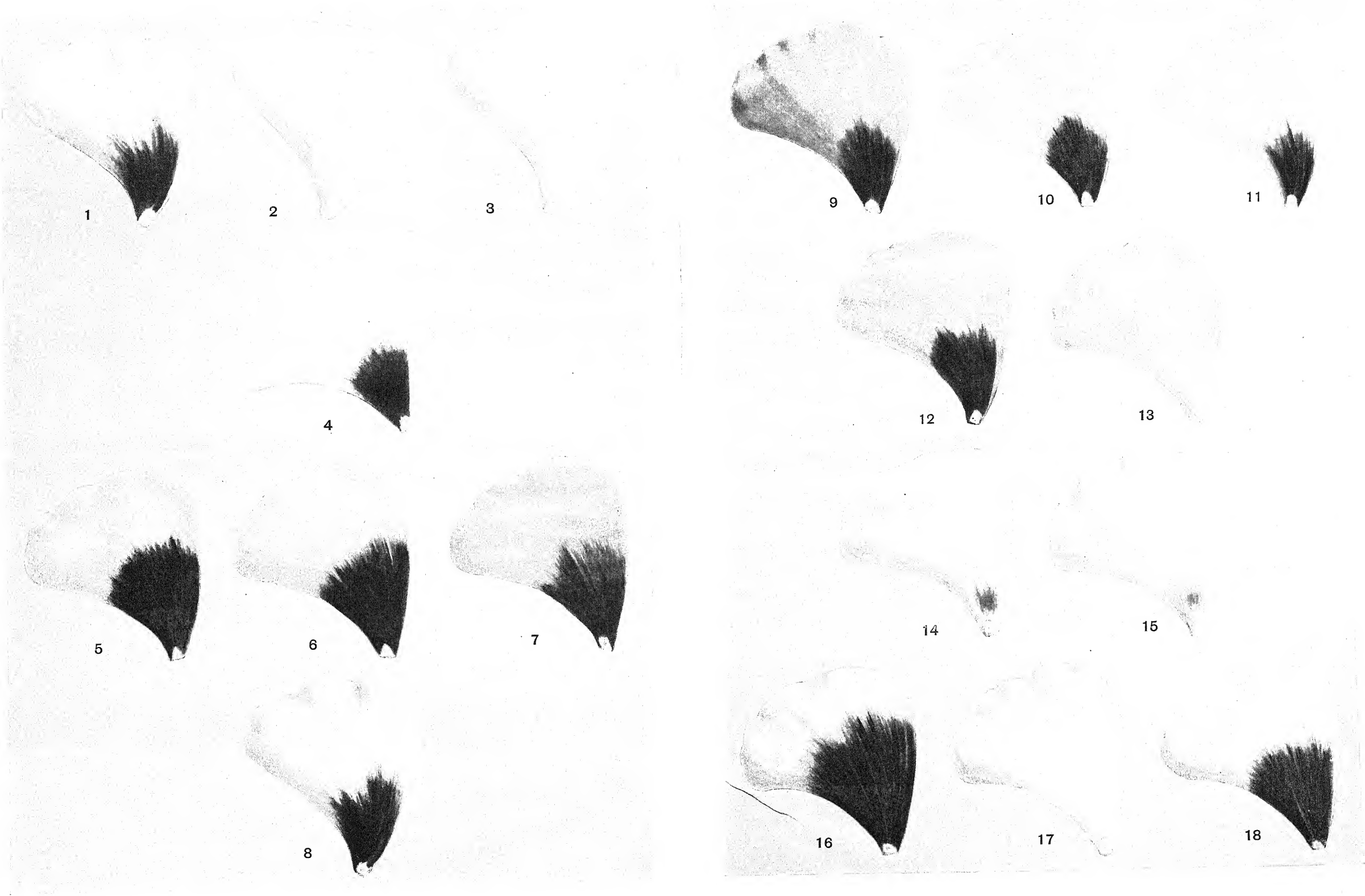
- Fig. 1. *G. arboreum* type A 8. Full petal spot ( $R_2^{AS} r_3^{OO}$ ).  
 Fig. 2. *G. herbaceum* type H 10. Spotless ( $R_2^{AO} r_3^{OO}$ ).  
 Fig. 3. *G. arboreum* type A 16. Ghost ( $R_2^{OS} r_3^{OO}$ ).  
 Fig. 4. *G. anomalum*.  
 Fig. 5. *G. anomalum* × A 8.  
 Fig. 6. *G. anomalum* × H 10.  
 Fig. 7. *G. anomalum* × A 16.  
 Fig. 8. Corolla colour: homozygous yellow as in fig. 1, heterozygous for yellow depressor  $Ydp$  (14,859).  
 Fig. 9. The petal colour effect of  $R_3^{GO}$ ; very intense gold ( $R_2^{OS} R_2^{OS} R_3^{GO} R_3^{GO}$ , homozygous  $Y_a$ ) (15,240).  
 Fig. 10. The petal colour effect of  $R_3^{GO}$ ; medium gold (heterozygous  $R_3^{GO}$ , homozygous  $Y_a$ ) (15,240 × 14,859).  
 Fig. 11. The petal colour effect of  $R_3^{GO}$ ; very faint gold (heterozygous  $R_3^{GO}$ , heterozygous  $Y_a^P$ ) (15,240 × N 5  $Y_a^P$ ).  
 Fig. 12. The petal colour effect of  $R_3^{GO}$ ; pink (heterozygous  $R_3^{GO}$ , homozygous  $Y_a$ , +  $Ydp$ ) (15,240 × 14,859).  
 Fig. 13. The petal colour effect of  $R_3^{GO}$ ; gold spotless ( $R_2^{AO} R_2^{AO} R_3^{GO} r_3^{OO}$ , homozygous  $Y_a$ ) (6144).  
 Fig. 14. Compound spot,  $F_1$  of A 16 × H 10 ( $R_2^{OS} R_2^{AO} r_3^{OO} r_3^{OO}$ ).  
 Fig. 15. Low grade compound spot, same constitution as fig. 14, from 14,350 × H 10 (5143).  
 Fig. 16. Large spot, from 15,305 × A 8 (19,018).  
 Fig. 17. Large ghost, from 15,305 × A 16 (18,947).  
 Fig. 18. Large compound spot, same constitution as figs. 14 and 15, from (*an.* × A 8) × H 10 (11,757).

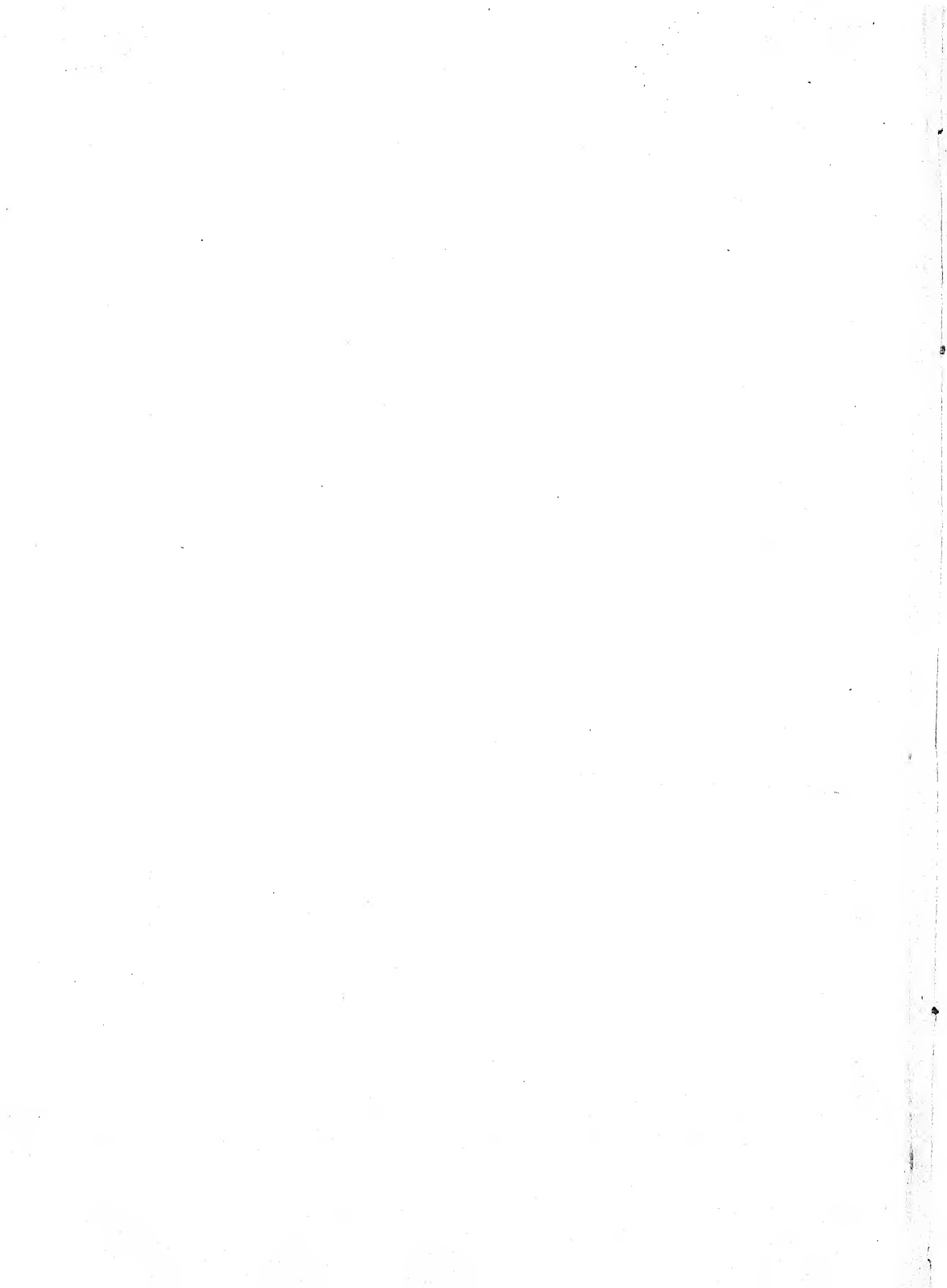
*Petal colour grades:* A 8, H 10 and A 16 are full yellow, grade 7. Fig. 8,  $Ydp$ , is grade 4.

*Spot size grades:* To obviate the necessity for printing a separate plate showing spot size grades, the range may be indicated as follows:

Spot size	Figure
15+	16
15	5
14	7
13	1
12	4, 9
10	3
6-7	14
4	15







# WOLF-DOG GENETICS<sup>1</sup>

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(With Plates 19-29 and Eight Text-figures)

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<sup>1</sup> [The MS. of this paper reached me about two years ago since when all attempts to get into touch with the author have failed. In view of the interest of the material and of the author's standing in the genetical world I have decided to take the responsibility of publishing it without his revision. For this reason many of the references in the text will not be found in the list at the end of the paper. I trust, however, that such references as the author had included will enable the interested student to track the rest. Ed.]

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## I. THE GENERAL PART

### (1) *Literature data*

THE most ancient record of the wolf-dog cross is that of Aristotle in the fourth century B.C. More exact information was given by Pliny (A.D. 23-79), who reported that the Gauls attached their bitches to trees in order to have them mated with wolves and so to obtain hybrids.

Later on, in the eighteenth and nineteenth centuries, a number of authors described such crosses. The majority of these crosses were not investigated in a scientific way. Usually they were either occasional experiments or measures directed towards the improvement of the local dog races (Kane (1856), Indian dogs; Seeman (1853), Lamare-Picquot (1860) and Hayes (1860), Eskimo dogs; Paget, Hungarian dogs, etc.). From numerous data of many authors (Kane, Seeman, Hayes, Lamare-Picquot, Paget, Mauduyt, Pallas, Cuvier, Hunter, Broca, Buffon, Flourence), Darwin (1868) was able to answer positively the question of the possibility of such a cross and of the fecundity of the hybrids.

Notwithstanding the great number of records, the data are haphazard and scrappy. Later, some further crosses, unfortunately for the most part insufficiently investigated, were carried out in zoological gardens and parks, where the wolf-dog crossing becomes in the nineteenth century in some sort "l'habitude de bon ton".

Roerig (1903) reported a series of such experiments carried out in 1830-70 at the "Jardin des Plantes" in Paris, at the Zoological Garden of Hanover and at that of Marseilles. Kühn (1887) conducted such a cross at the Zoological Garden in Halle, and Brehm (1909) at the Stockholm Zoological Garden. Similar occasional crossings were carried out before the Revolution at the Moscow Zoopark. (See the guide-books of the Moscow Zoological Garden.)

The above works did not give any important information on the wolf-dog question since genetical analysis was not applied.



This gap was partly filled up by the work of Bockelmann (1920) and the article by Lotsy (1922). The former analysed the nine wolf-dog skulls ( $5 F_1 + 4 F_2$ ) which had resulted from crosses conducted by Kühn (1887). Lotsy considered Bockelmann's data, but these are not very informative since his few hybrids are the progeny of several pairs of parents. This fact, combined with the small number of skulls, did not allow him to come to any definite conclusions. The results of crossings exhibited the same degree of heterogeneity in the size of the orbital angle both in the first (41–51°) and in the second (42–51°) generations.

Schäme (1922) gives supplementary materials towards the question of the possible segregation in  $F_1$  and  $F_2$  according to the skull measurements. He studied five  $F_1$  and four  $F_2$  skulls (probably the same as Bockelmann). Schäme's data are of a certain interest in connexion with segregation in the skulls of dogs crossed *inter se*, but they do not allow of a complete genetical analysis of the subject in question.

Certain data on single skulls of dog-wolf hybrids are scattered in the reports on special craniological investigations, but usually they are of no genetical interest.

The necessity of the study of more ample material on wolf-dogs from a genetical viewpoint is quite evident.

The question of other interspecific crosses of dogs is in the same position as that of their crosses with wolves.

Pallas (1780) and Hunter (1789) reported cases of dogs hybridizing with jackals; later on similar facts were reported by Saint Hilaire and by Blyth (cited from Darwin). Kühn (1887) obtained interesting data on the dominance of jackal characters in  $F_1$  and the heterogeneity of  $F_2$  (cited from Lotsy, 1922).

Later on Hilzheimer obtained a triple hybrid: wolf-jackal-domestic dog (Lotsy, 1922). Schäme (1922) observed segregation in the breadth of the skull in jackal-dog hybrids.

Numerous unsuccessful attempts to cross fox and dog have been made. Recently (1936) a successful cross by Heck is reported from Germany. We have so far no more precise data on the possibility of obtaining progeny from these fox-dog crosses. Nevertheless, a special kind of fox—the so-called *Pseudalopex (Canis) azarae*, which is not, indeed, a true fox, can be crossed with the dog, as shown by Krieg (1925). The latter described two litters from a female *P. azarae* and a male hybrid fox terrier.

Darwin (1868) reported the possibility of crossing Guiana dogs with the maikong, *Canis cancrivorus*, and apparently believed it possible to

cross American dogs with coyotes, *C. latrans*; but here American dogs are involved, and these differ considerably from the palaearctic races.

We see that in all cases of interspecific hybridization of dogs there was practically no attempt at any genetical analysis.

## (2) *The problem*

Our own studies have been specially concerned with the following questions.

First of all we were interested (a) in the possibility or impossibility of segregation in our interspecies crosses, (b) in the mode of inheritance of characters (in comparison with that observed in dogs), and (c) in the similarity of the segregation ratio to, or its difference from, the figures usually obtained in interracial crosses.

Such data should throw light on the problem of the homology of certain genotypical characters of the two species—wolf and dog. Besides interspecific crosses, certain investigators try to solve this general problem by comparing the phenotypical and genotypical properties of the species under investigation. But the results of such investigations become fully convincing only when accompanied by adequate genetical analysis.

Genetical literature contains few cases of a comparative genetical analysis of interspecific crosses among animals, e.g. (1) *Cavia porcellus* × *C. rufescens* (Detlefsen); (2) *Helix hortensis* × *H. nemoralis* (Lang); (3) *Drosophila melanogaster* × *D. simulans* (Sturtevant); (4) *Spinus spinus* × *Serinus canarius* (Promptoff) and some others.

The genetical analysis of wolf-dog hybrids should supply materials for the solution of the problem of the possibility of the wolf taking part in the origin of the dog, and of the practical problem of using the wolf to cross with our contemporary races of dogs.

Thus the principal problems in which I was interested while carrying out the analysis of the wolf-dog hybrids were as follows:

- (1) The applicability of the genetical laws to this interspecific hybridization;
- (2) The comparison of inheritance in wolf-dogs with that of dogs;
- (3) The homology of the factors of wolves and dogs;
- (4) The possibility of the wolf's taking part in the origin of the domestic dog;
- (5) The possibility of using the wolf for crossing with the contemporary races of dogs.

(3) *Material*

The original cross was carried out in 1923 at the Moscow Zoopark. A zonar-grey, wild-grey wolf male, caught wild, was crossed with a black mongrel sheep-dog female. This mating produced thirteen hybrid puppies. They were heterogeneous in respect of colour: seven were zonar-grey and six pure black (Pl. 19, figs. 1-3).

In November 1924 the black and the zonar-grey hybrids were mated with each other.

Later on some of the  $F_1$  hybrids were killed in order to obtain skulls, skeletons and fells. The rest of the hybrids selected for reproduction were mated with each other. Financial considerations did not allow the rearing of the hybrids on a larger scale.

We obtained 101 hybrids (Table 1) during 8 years—the period of the experiment (1923-30).

A considerable number of puppies died when young owing to unfavourable conditions. Certain questions remain unelucidated because of the small number of  $F_4$  puppies.

Table 1. *The number of wolf-dog hybrids*

Generation	No. of puppies
$F_1$	13
$F_2$	61
$F_3$	24
$F_4$	3
Total	101

Nevertheless, the number of the wolf-dogs examined was rather large—101 specimens—far exceeding the number of hybrids at the disposal of earlier investigators. The colour of seventy-three specimens was exactly examined. Thirty-nine skulls, among them twenty-nine hybrid ones, were carefully studied by me. Bockelmann and Schäme had had but nine hybrid skulls.

The rearing of a great number of hybrids and my uninterrupted work (1923-30) were possible through the kindness of a former Director of the Moscow Zoopark, Prof. M. M. Zawadowsky, to whom I offer my sincerest thanks.

## II. THE INHERITANCE OF THE COAT AND EYE COLOUR AND HAIR STRUCTURE

### (1) *Introduction*

Both in  $F_1$  and  $F_2$  hybrids there was observed definite segregation in coat colour, wool structure and eye colour (Table 2).

The chief colours of our hybrids were: zonar-grey, pure-black and brown-fulvous. The principal patterns: self-colour (uniformity), black and tan, white spots—markings.

There were also observed different degrees of the intensity of the pigmentation and different degrees of the development of tan markings.

The analysis of these crosses allows us to establish the mode of inheritance of characters studied, at least in a preliminary form.

It is almost beyond any doubt that the majority of the factors for these characters were introduced by the original dog whose genotype was unknown. Their segregation indicates the presence in wolves of factors allelomorphic to those of the dog.

We may now turn to our analysis of the separate characters.

(2) *Zonarity and the black and tan pattern (zonar, black, black and tan)*

It has already been mentioned that the  $F_1$  wolf-dog hybrids were of two kinds, zonar-grey and black. The ratio obtained (7 : 6) showed one of the parents to be heterozygous, and the dog was naturally suspected. Yet the results of crossing the  $F_1$  hybrids *inter se* showed that two black hybrids produced only non-zonar puppies, while two zonar-grey hybrids produced both zonar-grey and black (Table 2).

Evidently zonar-grey is dominant to black. Hence the wild wolf must have been heterozygous for black, or, to be more precise, in non-zonarity.

Cases of wild black wolves have been many\*times reported in zoological literature, especially by Ch. Darwin, and even before his time. They are undoubtedly the result of matings between two heterozygous wolves like that which was used for our experiments.

We may designate the gene for zonarity by **A** and that for non-zonarity (which is present in black wolves) by **a**.

We should now add that black  $F_1$  produced two kinds of black puppies: uniform black and black and tan (Pl. 20, figs. 4, 5).

The word "tan" in the dog designates either red (as if burned) or yellow spots in definite areas of the body: on the lower side of the muzzle, over the eyes, on the lower side of the chest, on the lower sides of the legs, etc.

Tan markings occurring in the black wolf-dogs should be distinguished from those which are met with in zonar-grey ones. In the latter the tan markings are light, slightly zonar, indistinctly limited, fusing into the surrounding background. They usually develop on the lower side of

Table 2. Segregation in the wolf-dog colour

No.	Parents		Progeny									
	Designation	Colour	Zonar			Black						
			Among them			Among them				Brown-fulvous with light eyes		
			In- tensified	Not in- tensified	Intensity unde- terminated	Total	Uniform	White spots	Black and tan	More precisely unde- terminated	Colour unde- terminated	Total progeny number
1	Wolf × dog	Zonar-grey × black	7	—	—	6	3	—	—	3	—	13
2	F <sub>1</sub> : no. 7 × no. 8	Zonar-grey with white band	17	4	9	3	—	—	3	—	3	23
3	F <sub>1</sub> : no. 9 × no. 10	Black	—	—	—	15	9	3	3	—	9	27
4	F <sub>2</sub> : no. — × no. —	Zonar intensified × zonar dilute	6	—	—	—	—	—	—	—	—	6
5	F <sub>2</sub> : no. 14 × no. 15	Black	—	—	—	4	3	1	—	—	—	4
6	F <sub>2</sub> : no. = × no. =	Zonar-grey intensified × black and tan (with a white spot)	1	—	—	3	—	—	3	—	—	4
7	F <sub>2</sub> : no. 3 × no. 12	Black and tan	—	—	—	8	—	—	8	—	—	8
		Total	31			39					12	85

the head, over the eyes, less distinctly on the legs. This peculiarity is a by-product of the zonar gene.

The shape of the true tan markings in our wolf-dogs is identical with those met with in Dobermann-pinchers, Gordon setters, etc., where they are definitely limited as either bright or pale yellow spots on the lower surface of the muzzle, on the neck, above the eyes, on the chest (two spots), on the lower interior part of the legs, around the anus and on the lower surface of the tail near the anus.

The presence of tan marking where the pattern is non-zonar is in wolf-dogs a recessive character.

Our crosses have led us to the conclusion that in wolf-dogs we are dealing with the multiple allelomorphic series

$$A > a > a^t,$$

where  $a^t$  denotes the presence of tan markings in non-zonar animals.

I may point out the *complete identity* of this allelomorph ( $a^t$ ) with that which I found in studying the genetics of the German sheep-dog and of Laika (Iljin, 1932), as well as that of the Dobermann-pincher (Iljin, 1931*a*, 1931*b*).<sup>1</sup>

On this hypothesis we may set out our various crosses as follows:

$$\begin{array}{l}
 P_1 \quad \frac{Aa}{\text{grey wolf}} \quad \times \quad \frac{aa^t}{\text{black dog}} \\
 F_1 \quad \frac{Aa + Aa^t}{\text{zonar-grey}} \quad + \quad \frac{aa + aa^t}{\text{black}} \\
 \hline
 \text{Wolf-dogs} \\
 P_2 \quad \frac{Aa^t \times Aa^t}{\text{zonar-grey}} \quad + \quad \frac{aa^t \times aa^t}{\text{black and tan}} \\
 F_2 \quad \frac{AA + 2Aa^t}{\text{zonar-grey}} + \frac{a^t a^t}{\text{black and tan}} \quad + \quad \frac{aa + 2aa^t}{\text{black}} + \frac{a^t a^t}{\text{black and tan}}
 \end{array}$$

(3) *The intensity of the red pigment in zonar wolf-dogs*

Among the zonar hybrids there may be noticed an evident heterogeneity in the intensity of the colour of the hair-band. A zonar hair is black (or brown, blue, etc.), with one or several yellow or white bands (zones) in its upper part.

Some of our hybrids had a very broad band of an intense yellow colour. They looked rust coloured or almost red with black stripes all over their bodies. Such "zonar-red" or "zonar rust" coloured specimens

<sup>1</sup> In my last work on the genetics of the Dobermann-pincher (1931*b*) there are reported facts which indicate the possibility of the existence of two kinds of tan markings similar in their phenotype, but different in their genotype. This fact brought us to the conclusion that in the dog a quadruple allelomorph  $A^t > A > a > a^t$  may possibly exist.

have been met with by zoologists among ordinary wolves, *Canis lupus*. We have met with such specimens among dogs (e.g. German sheep-dogs), though not often.

In other hybrids this zone is almost pigmentless, and looks either white or dirty grey. Such pigmentation is often met with in Laika-dogs (Iljin, 1932). The general colour of such a specimen appears as lead grey. Between these two extreme cases there exists an intermediary state of the band—a yellowish brown tint of a semi-saturated pigmentation.

Our wolf-dog crosses produced four zonar dogs with an intensive yellow band. They were born of the *inter se* crossed zonar specimens with a dirty white band. The ratio was as follows: four zonar with intensified band (zonar-rust) : four ordinary white-banded specimens : nine non-intensified zonar whose degree of intensity was not exactly determined. Two matings between zonar rust hybrids and ordinary white zonar ones gave six ordinary zonar puppies.

Here we have probably a recessive character, conditioned by a gene, which modifies the manifestation of the zonarity. Designating this gene with the letters **Int** (intensification of the zonarity) we have the allelomorphic pair

<b>Int</b>	>	<b>int</b>
Dirty white band		Bright yellow broad band

The yellowish brown band, similar to that which is often met with in German sheep-dogs, is evidently also a recessive character in relation to the dirty white band.

The relation of the yellowish brown to the bright yellow zones could not be established on our wolf-dogs. My former experiments on the hybridization of working dogs (German sheep-dogs and Laika) proved the genes of the yellowish brown zone and the bright yellow one to be an ordinary Mendelian pair (1932). The complete similarity of the inheritance in wolf-dogs and dogs of the dirty white and the bright yellow as well as of the greyish white and yellowish brown zones leads us, on analogy, to postulate similarity in the inheritance of other combinations of these characters.

In this case the following triple allelomorph would be established:

<b>Int</b>	>	<b>int<sup>m</sup></b>	>	<b>int</b>
Dirty white band		A moderate degree of the intensification of the band (yellowish brown band)		A high degree of the intensification of the band (bright yellow broad band)

(4) *The intensity of the red pigment in black  
and tan wolf-dogs*

Among our black and tan wolf-dogs could be observed differences in the intensity of the pigmentation of tan markings. In extreme cases these markings were bright rust coloured, intensely pigmented, though less than in Dobermann-pinchers. Intensely coloured tan markings behaved as recessive to paler ones. The lighter "tan markings" could be divided into two groups according to their phenotype, i.e. light yellow and almost pure white (Pl. 20, fig. 6). The behaviour of the latter is that of a dominant character in relation to the rust-coloured markings.

We have no complete material on the inheritance of the light yellow markings and therefore we can affirm only the existence of one pair of allelomorphs, viz.

$\text{Int}_1$  = greyish white markings and  $\text{int}_1$  = rust-coloured markings.

Probably in this case also we have a multiple allelomorphic series:

$\text{Int}_1$	$\text{int}_1^m(?)$	$\text{int}_1$
Greyish white markings	Light yellow markings	Rust-coloured markings

There evidently exists a phenotypical parallelism between the degree of the intensity of the tan markings and that of the intensity of the zonar hair band:

Markings	Band on a zonar hair
Greyish white	Dirty white
Light yellow	Yellowish brown
Rust-coloured	Bright yellow

The similarity between these two series of characters suggests that the intensity of the pigmentation of markings and that of the pigmentation of the band on the zonar hair are controlled by the same genes. If so the two series

and

$\text{Int}_1$	$\text{int}_1^m$	$\text{int}_1$
$\text{Int}_1$	$\text{int}_1^m$	$\text{int}_1$

must be regarded as identical.

At present we cannot give a definitive answer to this question.

Mention may here be made of analogical conditions in rabbits. Pap (1921) found that the intensity of the colour of yellow marks in black and tan rabbits varies between rather wide limits. He considers this phenomenon as due to the presence of polymeric multiple dominant genes which reduce the intensity of the pigmentation of the marks, viz.

$$Y_1 \ Y_2 \ Y_3 \dots > Y_1 \ Y_2 \ Y_3 \dots$$



Nachtsheim (1929) points out that the cross between the black and tan rabbit with light-coloured tan markings and the so-called "hare rabbit" (zonar with yellowish red tint) produces an intensification of the tan pigmentation in the black and tan progeny. Pap also points out the similarity in the inheritance of the yellow pigmentation in the "hare" and the black and tan rabbits.

(5) *Brown-fulvous colour and light iris*

Two black  $F_1$  wolf-dogs, female "Disa" and male "Mak", were crossed and produced a progeny among which were three puppies of a peculiar brown-fulvous colour. When just born these brown-fulvous specimens were difficult to distinguish from the black ones. Later on the difference in their colour grew more marked. It is even noticeable in the photos (Pl. 21, figs. 7-9). This brown-fulvous colour somewhat resembles the brown colour of Newfoundland dogs, though it is somewhat duller (lustreless) than in the latter.

The eyes of these three wolf-dogs were light with a whitish blue iris (Pl. 21, fig. 8), somewhat like the eyes of marbled Great Danes (Iljin, 1926*b*, 1928). The peculiar colour of the eyes is probably a by-product of a gene for brown wool (or at least of a gene linked with the former), since it occurred only in the presence of the brown colour.

The precise number of brown-fulvous puppies could not be established because of the death of some at an age when the phenotype in question could not be distinguished from that of the black ones.

For segregation in this cross see Table 3.

Table 3. *Black  $F_1$  inter se*

	Black	Black and tan	Brown	Brown and tan	Phenotype undeter- mined	<i>n</i>
Obtained	12	3	3	—	9	27
Expected	10.13	3.37	3.37	1.13	—	18
Difference	1.87	0.37	0.37	1.13	—	—

Since the manifestation of the brown-fulvous colour was independent of other characters, there must exist an independent allelomorphic pair, viz. black (**B**) and brown-fulvous (**b**).

This pair is identical with that long ago established by Lang for sporting dogs (Lang, 1910; Iljin, 1926*a*) and more recently by N. A. Iljin (1931*a*) for Dobermann-pinchers (see Table 5).

(6) *White spots*

Both the  $F_1$  hybrids crossed *inter se* and the  $F_2$  hybrids crossed *inter se* produced, besides the ordinary self-coloured puppies, specimens with white spots. White spots occurred in the form of relatively well-outlined white markings of various dimensions in different specimens.

At the same time there could be noticed a strict invariability of the localization of these white markings like that of the white markings in ordinary dogs: *the depigmentation of the wool cover begins in strictly definite places and only in these places*. The examination of our wolf-dogs showed a series of specimens with white spots: in some of them the depigmentation is only in its initial stage and begins with a definite point only, in others it spreads over different areas of the adjacent region. A small white spot in one specimen may be connected by a series of uninterrupted intermediary forms with big white spots in some other specimens. These data confirm the regularity of spot spreading which I had established on guinea-pigs. White spots are the result of the process of the depigmentation which begins at definite points termed "points of origin of depigmentation" (Iljin, 1928).

I have established the presence of such points of origin of depigmentation in dogs (Iljin, 1928, 1932), and in particular in Dobermann-pinchers (Iljin, 1931a), in Caucasian sheep-dogs (unpublished), etc.

The white markings in dogs were localized in the following places: the chest, the tips of the toes of the fore- and hindfeet, and the lower half of the feet ("stockings").

We have no room for a detailed analysis of the inheritance of this character, but we wish to point out that specimens without white spots may certainly produce specimens with white spots. Therefore I consider myself justified in postulating the allelomorphic pair **S**, **s**, where **s** is the recessive character of the presence of white spots and **S** the absence of white spots (self-colour). The same allelomorph has been established for other mammals, in particular for dogs.

The genes for the white spots in our wolf-dogs were probably received from the dog. However, the presence of these genes in wolves is also possible, since naturalists have sometimes (though rarely) recorded wolves with white spots on the chest. Our wolf was probably **SS** and the original dog **Ss**. This would account for the segregation of white spotting in  $F_2$  and  $F_3$  hybrids, though, of course, there may be another explanation.

(7) *The inheritance of colour in wolf-dogs in comparison with that in dogs*

Let us summarize the allelomorphs whose existence explains the segregation in our wolf-dogs (Table 4), and compare them with data obtained by different authors for dogs (Table 5).

The examination of Table 5 shows a complete analogy in the inheritance of colour in wolf-dogs and in different races of dogs. *The segregation in the colour of the wolf-dog hybrids proceeded in the same way as in the progeny of dogs crossed with dogs. Characters, recessive in dogs, are recessive in wolf-dog hybrids; characters, dominant in dogs, are dominant in wolf-dogs. The genes of dogs, introduced into crosses, behave in just the same way as when they meet with their normal allelomorph.*

Table 4. *Colour factors in wolf-dogs*

Genetical symbols	Hereditary characters		
I. $A > a > a^t$	Zonar	> non-zonar	> the presence of tan markings in non-zonar specimens
II. $Int > int^m > int$	Dirty white (dilute) band in zonar hair	> yellowish brown (moderately intensified) band in zonar hair	> bright yellow (intensified) broad band in zonar hair
III. $Int_1 > int_1^m(?) > int_1$	Greyish white (dirty white) tan markings	> light yellow tan markings	> rust-coloured tan markings
IV. $B > b$	Black colour	>	brown-fulvous colour (with light iris)
V. $S > s$	Self-colour (without white spots)	>	white spotting

Evidently the wolf possesses genes which are normal allelomorphs for the dog genes of the II, III, IV and V allelomorphs.

It seems very probable that the wolf possesses genes of the II, III, IV and V allelomorphs not only homologous, but *identical* with the corresponding genes of the dog.

With regard to gene *a*, the cross of the wolf *Aa* with the dog *aa* and the subsequent breeding of the *F*<sub>1</sub> and *F*<sub>2</sub> *demonstrated its identity* in the wolf and the dog. Hence these two species *possess certain identical genes in the same linkage groups*. They must therefore possess identical loci, which fact is a new illustration of Vavilov's law of homologous series, a new striking confirmation of Darwin's idea about parallel variation.

The possibility of the presence in wild wolves of certain colour factors is as follows:

The non-zonarity factor *a* is sometimes met with in wolves, resulting in black-coloured animals.

White markings in wild wolves have been many times recorded by naturalists.

Table 5. *The wolf-dog factors in comparison with the dog factors*

Wolf-dogs. Allelomorphs	Dogs		
	Allelomorphs in our symbols	Races	Authors
I. $A > a > a^t$	$a > a^t$	Spaniels Dachshunds Griffons	Barrows & Phillips, 1915 Ibsen, 1916 Anker, 1925 Little, 1934 Iljin, 1932
	$A > a > a^t$ $a > a^t$	German sheep-dogs Dobermann-pinchers Gordon setter $\times$ Irish setter	Iljin, 1931a, 1931b, 1932 Iljin, 1932
II. $Int > int^m > int$	$Int > int^m > int$	German sheep-dogs Siberian Laikas	} Iljin, 1932
III. $Int_1 > int_1^m(?) > int_1$	$Int_1^m > int_1$ $Int_1 > int_1^m$	Dobermann-pinchers Siberian Laikas	
IV. $B > b$	$B > b$	Newfoundland dogs Pointers Spaniels Great Danes Dachshunds Dachshunds and naked dog Dobermann-pinchers Siberian Laikas and Rotweilers Hounds	Lang, 1910 Little, 1914 Barrows & Phillips, 1915 Little & Jones, 1919 Anker, 1925 Plate, 1925, 1929 Iljin, 1931a, 1931b, 1932 Iljin, 1932 Steiger, 1936
V. $S > s$	$S > s$	Hounds Great Danes  Dachshunds and naked dog Greyhounds Dobermann-pinchers German sheep-dogs, Riesen Schnauzers, Rotweilers, Aire- dale terriers Siberian Laikas, Caucasian sheep-dogs English bull-dogs, Newfound- land dogs Russian wolfhounds (borzoi), Russian hounds Setters: English, Irish and Gordon Boxers  Terriers Collies (always homozygous for s)	Lang, 1910 Little & Jones, 1919 Iljin, 1926b, 1932 Plate, 1925 Warren, 1927 Iljin, 1931a, 1931b Iljin, 1932  Iljin, 1932 Dahl & Quelprud, 1937 Hirschfeld, 1933 Mitchell, 1935

About  $A > a > a^t$ ,  $B > b$ ,  $S > s$  and others. See also the reviews and summarizing works: Wright, 1918; Haldane, 1927; Iljin, 1932; Dawson, 1937.

The existence of variations in the intensity of the general colour of the wolf, recorded by Brehm and by other zoologists, is a fact in favour of the possibility of the presence of genes which belong to our allelomorphic

series  $\text{Int} > \text{int}^m > \text{int}$ . The existence of the rust-zonar (the so-called "red") wolves is a confirmation of the manifestation of the gene  $\text{int}$ .

Uniformly self-white wolves are sometimes (though very rarely) met with wild (Taenzer, 1923-5). Such wolves have been recently (1925) discovered in the southern "Taiga" (wild conifer forests) of the Eniseisk region in the U.S.S.R. These facts suggest the existence in wolves of the recessive gene for albinism.

We must, however, point out that Kandern (1905) considers the Greenland and North American white wolves as members of the species *Canis occidentalis*, not *C. lupus*. Stroman (1925) has described some cases of the albinism in the American *C. latrans*.

In the so-called "blue" wolves the colour is not blue but zonar black with a dilute black band, giving the impression of an ashen blue shade. Such "blue" wolves have been caught (1928) in the northern region of Tobolsk in the U.S.S.R. Perhaps we have in this case a gene which reduces the intensity of the black pigment ( $c^d$  - ?), or perhaps even a gene for the brown colour ( $b$ ) discovered in our wolf-dogs.

Our original wolf was undoubtedly heterozygous in  $A$ . Moreover, he was probably also heterozygous for  $\text{Int}$ , because it is unlikely that the bright yellow intensified broad band gene was introduced by the dog. This gene accounts for the zonar-rust-coloured hybrids of the type of "rust" ("red") wolf. In this case the genetic formula of our wolf must have been  $Aa \text{Int int}$ , though the possibility of its having been  $Aa \text{Int Int}$  is not excluded.

On the basis of the above data we may suppose the formulae of our original specimens to have been as follows:

$$\begin{array}{l} \text{Aa Int int (int}_1 \text{Int}_1) \text{BB SS} \\ \text{or Aa Int Int (Int}_1 \text{Int}_1) \text{BB SS} \end{array} \times \frac{\text{aa}^t \text{int}^m \text{int}^m (\text{int}_1^m \text{int}_1) \text{Bb Ss}}{\text{dog}}$$

wolf

#### (8) Curly wool

Some of our hybrids had soft, curly wool. For instance, the no. 3 in the  $F_2$  generation, named "Els", had soft wavy wool which could be described as curly since it resembled the wavy wool with long curls sometimes met with in German sheep-dogs. The fact of normally woolled specimens producing curly hybrids shows this character to be recessive (presuming it to be hereditary).

## III. THE INHERITANCE OF EXTERNAL CHARACTERS

(1) *Body size*

According to Brehm the height of the wolf in the shoulders reaches 85 cm. According to Satunin (1895) the height of the wolf of the Moscow district reaches 84 cm. Ogniev (1931) reports the height at the croup of two wolves from the Voroniej district, U.S.S.R., to be 80 and 72 cm.

The height of the wolves we measured at the Moscow Zoopark was 57.5, 63 and 63.5 cm.

We have no data about the dimensions of the original dog, therefore we give corresponding figures for German sheep-dogs—our measurements of Moscow dogs in 1931. These range from 48 to 68–72 cm. The average height of the males is  $63.9 \pm 0.55$  cm. and of the females  $61.5 \pm 0.35$  cm.

The length of the wolf's body is given in Brehm's measurements as that from the nose tip to the tail tip. On this primitive method it was 160 cm. (the tail – 45 cm.; the head, neck and body 115 cm.).

Ogniev (1931) gives the following figures for two wolves of the Voroniej district: adult male, tail length (with terminal hairs), 51 cm.; body length (from the nose tip to the base of the tail), 125 cm.; adult female, tail length, 46.2; body length, 119 cm.

The corresponding figures for the German sheep-dog may be obtained by summing up the average figures of measurements of the length of the body from the withers to the base of the tail and of those of the head length and the neck length. These figures for the males are:  $48.5 \pm 0.5$  cm., the length of the tail; 106 cm., that of the body, neck and head. For the females they are:  $46.6 \pm 5$  cm., the length of the tail; 100.5 cm., that of the head, neck and body (our measurements in 1931).

The *skull length* in wolves<sup>1</sup> ranges according to Studer (1901) from 186 to 243 mm. The "normal" length apparently begins with 197 mm. The skull length of ordinary sheep-dogs, German sheep-dogs in particular, is according to Studer 164–179 mm.

The *body weight* of the wolf according to Brehm reaches 40–50 kg. The maximal weight according to Ogniev (1931) is 69–78 kg. The weight of an adult German sheep-dog ranges from 21.2 to 40.6 kg. The average for the males is 30.7 kg. and for females 25.1 kg. (as measured by Mrs L. J. Levkovich at our laboratory).

A number of other measurable characters gives for the wolves higher figures than for German sheep-dogs. Later on (Table 6) we give the

<sup>1</sup> The maximal skull length is according to Ogniev (1931): ♂♂ 268–285, ♀♀ 251–268. In this case the figures are other than in craniological material usually met with. This fact may be attributed either to other methods of the measurement or to unusual material.

dimensions of four wolves which were measured in Moscow Zoopark on our suggestion. The measurements were carried out on living specimens.

Table 6. *Chief external dimensions of four wolves (all males) in the Moscow Zoopark (in cm.)*

Nos. 1 and 2, caught in normal conditions; nos. 3 and 4, born in Moscow Zoopark				No. 1	No. 2	No. 3	No. 4
No. and name ... ..				"Volt-chock"	"Grishka"	"Lobaty"	"Argo"
Date of measurement ... ..				Feb. 1929	Feb. 1929	Feb. 1929	Feb. 1929
Notes ... ..				Measured living	Measured when dead	Measured living	Measured living
No.	Dimensions						
1.	The length of the forehead			14.5	14.1	13.7	12.7
2.	The length of the muzzle			13	13.9	14	14.5
3.	The length of the head (measured with beam compasses)			27.1	26.6	27.2	23.3
4.	The length of the head (measured with a tape-measure)			29	29.5	29	26.5
5.	The breadth of the skull across the processii supraorbitales			5.2	4.5	5.2	6.5
6.	The breadth of the skull across the fossae temporales			5.8	5	5.8	6.8
7.	The distance between the bases of the ears			7.3	8.1	8.5	8.7
8.	The length of the ear			12.5	14	13	11.5
9.	The breadth of the ear			11.5	11.5	11.5	12
10.	The length of the neck			27.7	27.3	21.9	—
11.	The circumference of the neck			—	44	45	45
12.	The length of the body (measured with a Lidtin's stick)			65	—	65.5	56
13.	The length of the body (measured with a tape-measure)			49	63.5	54	53.5
14.	The height at the withers			57.5	—	63.5	63
15.	The height at the sacrum			59	—	62	59
16.	The "shoulder line"			21.5	—	20	17.5
17.	The depth of the chest			22	26	24	23.5
18.	The circumference of the chest			68	71	80	75
19.	The circumference of the groin			50	52	65	56
20.	The length of the tail			52	44	36	39
21.	The length of the shoulder			32.5	33.5	37	37
22.	The length of the foreleg			34.5	36	35.5	34.5
23.	The length of the hindleg			20	19	20	20
24.	The length of the shank			28	30	30	30
25.	The breadth of the chest			17.5	—	18.5	16.5
26.	The height of the back			59	—	63.5	59
27.	The breadth of the croup			10.5	—	9	9
28.	The side length of the croup			26	28.5	29	26
29.	The side-long length of the body			67	68	62	61
30.	The breadth of the forehead			10.9	—	11.9	—
31.	The side length of the head			23.1	22.8	21.2	—

These figures may be compared with the corresponding figures (Table 7) obtained by our measurements on German sheep-dogs.

These data, as well as the review of the above-cited literature and of our data (Table 8), show that the dimensions of the sheep-dog are smaller than those of the wolf. Though we have no exact data about the

dimensions of the original dog of our cross, there can be no doubt that it was smaller than the wolf. The dimensions of the wolf-dog hybrids of the first generation are similar to those of middle-sized or rather larger dogs (Table 9). They are probably intermediary forms as to their dimensions.

In the second generation of wolf-dogs there was observed a definite segregation for the body size. The majority of the hybrids are middle-

Table 7. *Chief external dimensions of German sheep-dogs (in cm.)*

No.	Measurements	Adult males (average from 50 individuals)				Adult females (average from 53 individuals)			
		$M \pm m$		Limits		$M \pm m$		Limits	
				Min.	Max.			Min.	Max.
1	Length of the forehead	12.58	0.17	10	15	12.24	0.16	10	14
2	Length of the muzzle	13.52	0.11	12	15	12.51	0.12	12	15
3	Length of the head (measured with Stangen-Zirkel)	25.70	0.17	22	28	24.34	0.15	21	26
4	Length of the head (measured with a tape-measure)	27.60	0.20	24	31	26.34	0.18	23	30
5	Breadth of the skull across the processi supraorbitales	4.68	0.09	4	6	4.47	0.09	3	6
6	Breadth of the skull across the fossae temporales	6.48	0.09	5	7	5.93	0.10	4	8
7	Distance between the bases of the ears	7.40	0.12	6	9	7.19	0.07	6	9
8	Length of the ear	12.00	0.17	9	15	11.48	0.12	10	13
9	Breadth of the ear	11.38	0.15	9	14	10.72	0.10	10	12
10	Length of the neck	21.18	0.28	18	27	19.74	0.21	17	24
11	Circumference of the neck	42.24	0.34	36	48	38.68	0.29	33	43
12	Length of the body (measured with a Lidtin's stick)	67.68	0.64	45	74	65.07	0.53	56	72
13	Length of the back	59.42	0.53	52	67	58.00	0.45	49	67
14	Height at the withers	63.88	0.55	48	71	61.49	0.35	57	68
15	Height at the sacrum	62.94	0.55	49	71	60.76	0.36	56	68
16	The "shoulder line"	20.64	0.25	17	25	19.60	0.29	16	27
17	Depth of the chest	23.50	0.43	17	29	22.04	0.33	17	27
18	Circumference of the chest	72.52	0.52	60	79	68.28	0.45	57	74
19	Circumference of the groin	55.53	0.63	48	65	51.24	0.60	41	63
20	Length of the tail	42.72	0.45	37	52	40.74	0.42	33	49
21	The distance between the withers and the "elbow"	31.06	0.25	28	35	29.94	0.22	26	34
22	Length of the foreleg	34.82	0.29	30	38	33.02	0.21	30	36
23	Length of the hindleg	19.08	0.26	15	29	18.17	0.13	16	20
24	Length of the shank	28.98	0.44	20	32	26.55	0.43	20	37

sized (Pl. 20, figs. 4, 5, etc.), like  $F_1$ . Besides these middle-sized hybrids there segregate out both larger and smaller specimens. Particularly instructive is the presence in the same litter of the huge male, no. 42, "Boorij" (brown), and the small female, no. 43, "Malyska". The former is twice as large as the latter (Pl. 21, fig. 8 and Pl. 23, fig. 12).

## (2) The body form, the type of the body frame

Unfortunately our material did not allow us to come to precise conclusions about the body frame of the wolf-dogs. A number of



external characters in the constitution of animals depend upon external conditions. The conditions under which our hybrids were kept were far from ideal, and have often led to rachitic and other changes in some of the animals.

It is well known that the body frame of the dog is very like that of the wolf. In many cases the wolf cannot be distinguished from the dog even at a short distance and vice versa (Richardson, 1829; Kane, 1856; Hayes, 1860; Darwin, 1868, etc.).

According to Brehm the external differences between the wolf and the dog are as follows:

The body of the wolf is longer (?) and the outline of its back tends to sink more than that of the dog. The position of its shoulder-blades is

Table 8. *Chief external dimensions of the wolf and the German sheep-dog*

No.	Measurements	Wolf	German sheep-dog
1	Height (at the withers)	85 cm. (Brehm) 84 cm. (Satunin) 57.5, 63.0 and 63.5 cm. (our data)	Limits, 48-68-72 cm. The average for males, $63.9 \pm 0.55$ cm.; for females, $61.5 \pm 0.35$ cm. (our data)
2	Height at the sacrum	72 and 80 cm. (Ogniev) 59 and 62 cm. (our data)	Limits, 49-71 cm. The average for males, $62.9 \pm 0.55$ cm.; for females, $60.8 \pm 0.36$ cm. (our data)
3	Length of the head + length of the neck + length of the body	115 cm. (Brehm) 125 cm. (male) 119 cm. (female) 105.7 cm. (our data)	Males, 106.0 cm. Females, 100.5 cm. (our data)
4	Length of the tail	45 cm. (Brehm) 51 cm. (male) 46.2 cm. (female) 36-52 cm. (our data)	Males, $48.5 \pm 0.5$ cm. Females, $46.6 \pm 0.5$ cm. (our data)
5	Length of the skull	186-243 mm. (Studer)	164-179 mm. (Studer)
6	Weight	40-50 kg. (Brehm) Maximum, 69-78 kg. (Ogniev)	21.2-40.6 kg. Average for males, 30.7 kg. Average for females, 25.1 kg. (our data)

more oblique, the humero-scapular joint being situated nearer to the thorax. From before, the chest is almost invisible behind the joints, the "elbows" being turned inwards and pressed to the chest. The legs are quite straight with pads slightly turned outwards. The dog's scapulae are steeper; the joint pits for humerus are placed nearer to the hindpart of the thorax; its shoulders are shorter than those of the wolf.

This description does not lead us far, but we may observe that the majority of the characters proper to the wolf when met with in dogs are considered as the consequences of unfavourable environmental conditions.

At any rate such characters as narrow chest, chest pressed between scapulae, and feet turned outwards, are reckoned among the negative characters in describing the externals of a dog.

Table 9. *Chief external dimensions of six wolf-dog hybrids (in cm.)*

No. and name ...				<i>F</i> <sub>1</sub> no. 8 "Mam- sel", zonar Female	<i>F</i> <sub>1</sub> no. 7 "Sku- kum", zonar Male	<i>F</i> <sub>2</sub> no. 42 "Boorij", brown with white eyes Male	<i>F</i> <sub>2</sub> no. 3 "Els", black Male	<i>F</i> <sub>2</sub> no. 26 "Usnai", black and tan Male	<i>F</i> <sub>2</sub> no. 12 Black Female
Sex	...	...	...	Female	Male	Male	Male	Male	Female
Age	...	...	...	5 yr. 7 m.	5 yr. 7 m.	1 yr.	4 yr.	3 yr. 3 m.	5 yr. 2 m.
Date of measurement				Feb. 1929			Mar. 1930		
No.	Dimensions								
1	Length of the forehead			11.8	13.2	13.7	12	13	11.1
2	Length of the muzzle			13.1	12.8	12.4	11.7	13.2	11.6
3	Length of the head (measured with Stangen-Zirkel)			23.5	24	24.6	23	25	22.5
4	Length of the head (measured with a tape-measure)			25.5	27	26	24.5	27	24.5
5	Breadth of the skull across the processi supraorbitales			4.9	5.4	5.9	5	5.6	5.4
6	Breadth of the skull across the fossae temporales			6.1	5.9	6.7	6	6.1	5.8
7	Distance between the bases of the ears			6.8	8.7	7.1	6.9	6.5	5.3
8	Length of the ear			13	13.5	14.5	14.5	14	13
9	Breadth of the ear			12	11	13	10.5	12	9.5
10	Length of the neck			20.3	23.8	21.4	18.3	22.4	22.7
11	Circumference of the neck			37	41	42	37	39	33
12	Length of the body (measured with a Liddin's stick)			57.5	54	61.5	57	57	59
13	Length of the body (measured with a tape-measure)			47	49	63	51	48	50
14	Height at the withers			53.5	59	63.5	56.5	54	—
15	Height at the sacrum			53	57	58	54.5	53	—
16	The "shoulder line"			17.5	19.5	21.5	18	19	16
17	Depth of the chest			15.5	21.5	25	24	23	22
18	Circumference of the chest			59	68	68	63	61	58.5
19	Circumference of the groin			27	50	59	47	47	—
20	Length of the tail			35	43	56	46.5	50	—
21	Length of the shoulder			29	31	35	29	28	27
22	Length of the foreleg			33	33	35	29	33	29
23	Length of the hindleg			18	17.5	18	14.5	17	16.5
24	Length of the shank			24	28	30	24	27.5	25.5
25	Breadth of the chest			14.5	19	19	14.5	15.5	—
26	Height of the back			94	54.5	59	—	—	—
27	Breadth of the croup			8	9.5	8	9.8	11.6	—
28	The side length of the croup			26	25	28	25	26	24
29	The side-long length of the body			52	55.5	62	55	57	51.5
30	Breadth of the forehead			10.1	10.8	9.6	10.4	11.9	9.2
31	The side length of the head			18.9	19.1	20.8	18.8	20.2	18.9

Some other characters, as for instance obliquely placed shoulders and straight legs, are proper to every well-built service and working dog. Hence these cannot be considered as characters in which the dog differs from the wolf.

In Brehm's list of the characters specific for the wolf (Taenzer, 1923-5, also cites this list) there are none essentially differentiating the wolf from the dog.

There seem to be very few or even *no real* external differences between the wolf and the working dog. There exist but modificatory changes under unfavourable external conditions of the development. A comparison of the externals of our four measured wolves with those of German sheep-dogs confirms this conclusion, though a final answer could be obtained only by means of measuring a great number of living wolves, which is rather impracticable.

Besides the comparison of single measurements of the wolves with those of the German sheep-dogs we have carried out a biometrical comparison of their external characters. The method applied was that of an objective evaluation of the externals of the German sheep-dog (worked out for dogs by Iljin in 1931), and was carried out on Heinke's method. Three of the wolves measured proved similar to the first group of German sheep-dogs (ranked highest for externals). The fourth could be regarded as an ill-proportioned dog.

At any rate the type of the body frame of the wolf is very like or even identical to that of the German sheep-dog. This is in accordance with the hypothesis of morpho-phylogenetic relationship of German sheep-dogs to wolves, and with their being placed in the same zoological group.

The absence of any essential difference between the body-frame type of the wolf and that of the German sheep-dog explains the absence of difference in the frame type of our  $F_1$ ,  $F_2$  and  $F_3$  hybrids, notwithstanding the apparent heterogeneity of their forms (besides the above-mentioned, see also Pl. 23, figs. 13, 14) and dimensions. (The animals obtained were large, middle-sized, small; some of them had sufficiently, while the others insufficiently, developed "physiologically conditioned" characters, etc.)

Therefore I feel justified in supposing that the wolf may be crossed with the German sheep-dog without affecting the external characters of the latter. Such crosses might be carried out in order to obtain service dogs (military, police or detective dogs, etc.). At the same time such a cross would afford the opportunity of obtaining many new combinations by selection, and also of utilizing such genes for vitality and resistance as had accumulated in wolves over a long process of natural selection.

*(3) Gait and footprints*

Our viewpoint is that the majority of the external characters proper to the wolf are those which occur *in dogs* as the result of unfavourable conditions of their development, and this leads us to some interesting conclusions on the normal gait of the wolf.

In zoological literature there exist many reports about the differences in the gaits of the dog and the wolf.

The wolf when walking or trotting places its hindlegs in the prints of its forelegs. The legs move forward in a straight line and the trail stripe they leave is characteristically straight (Text-fig. 1). The trail of the fox is similar.

The dog walks "cross-wise" (cross-cross): when walking or trotting it places its hindlegs between its forelegs and leaves an undulating sinusoid-like trail.

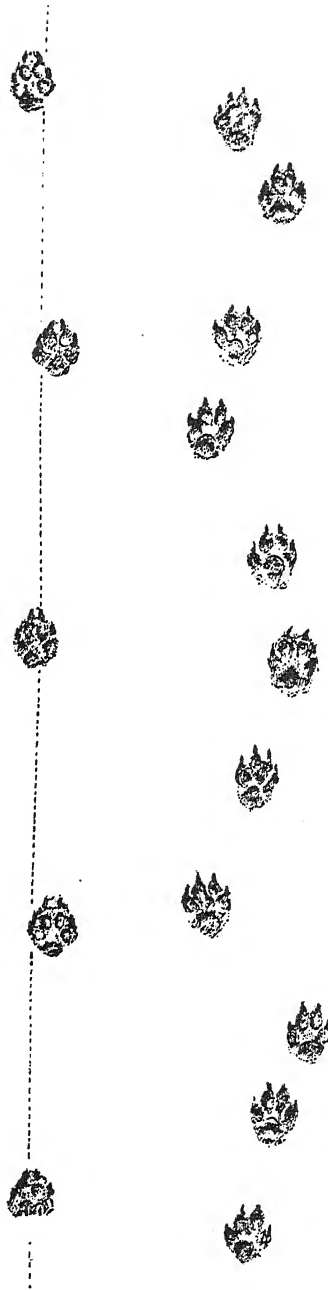
Only in galloping are the movements similar; both animals leave double footprints, because they thrust their hindlegs in front of the forelegs, placing the latter apart.

The description of the wolf given in § 2 affords an explanation of the peculiar wolf gait. It is the consequence of the wolf's legs being pressed to its chest and its paws turned outwards. A certain sinking depression of the back (in case it is present) must be connected with this character. The depression of the back is also met with in dogs with paws turned outwards.

Our hypothesis is confirmed by the fact that the steps of the wolf are rather short. Notwithstanding its large dimensions in comparison with middle-sized hounds, the latter's steps are almost as long as those of the wolf. It becomes obvious when one observes the gait of the wolf and that of the hound, and may be confirmed by measuring their footprints.

Thus Teuwsen & Schulze (1901) give parallel pictures of the wolf's and hound's trails. The measurements of the reduced picture give 9 cm. for the step length of the wolf and 8 cm. for that of the hound. The difference in length is only 11–12%, while on the difference in the height between the hound (50–55 cm.) and the wolf (75 cm.) it should reach 36% under the condition of an equal development of the extremities and of the angles under which they are attached to the cinguli membrorum.<sup>1</sup>

<sup>1</sup> Mrs E. Iljin supposes that, on the contrary, the wolf makes larger steps than the dog (about 0.5 m. long). Probably it takes place in single cases.



Text-fig. 1. Schematic picture of footprints of a wolf (left) and those of a hound (right) according to Teuwsen & Schulze (from Taenzer).

Of course it is impossible to affirm that the peculiar gait of the wolf is the consequence of the above causes only. At any rate the specific development of certain characters of the wolf's externals play a certain role in the formation of its peculiar gait.

I have often observed *working-dogs* (German sheep-dogs, Laikas) *with an almost typical wolf gait* as a consequence of a wrong development of the leg set, and of the wrong relation of the angles between the extremities and the body.

In our wolf-dog hybrids, in consequence of the unfavourable environmental conditions, there were observed different types of deviations from the norm in the development of their extremities. They had, for instance, legs turned outwards, "cow-like position" of the legs, the "French" position of the legs, etc. Therefore I do not feel justified in coming to any definite conclusions about the character of their gait, since this character may be conditioned by the type of body frame which may not be hereditary.

With regard to the footprints Anna Tracy (1930) believed it possible to establish the following differences between those of the dog and of the wolf; in wolf-prints the two middle claws are drawn together, in dog-prints they are set apart; the claws of the wolf are thicker than those of the dog, and its print is longer, narrower and more hairy. The toe-cushions are harder and therefore their prints are more distinct. Taenzer (1923-5) does not agree with Tracy's opinion. He cites Teuwsen-Schulze and Brehm, and points out that according to his own observations the claws on the forepaws of the German hounds are set closer to each other than on those of the wolf.

Though the wolf's print shows greater length, compactness and distinctness in comparison with the rounder outlines and indistinctness of that of the dog, the difference is relative only, nor did observations on about fifteen among our wolf-dogs enable us to establish any typical differences.

#### (4) *Tail*

The tail set is one of the principal characters in which the dog differs from the wolf, a fact already noted by Linnaeus.

The wolf has a hanging log-shaped straight tail. Dogs have tails of different shapes, ranging from straight to strongly curved, the latter in typical cases being slightly turned or curved to the left (Linnaeus). Blasius (1857) and Haacke (1903) persistently affirm that the dog's tail

is always turned to the left.<sup>1</sup> This is not so. I have seen dogs of different races whose tails were turned to the right. Nehring (1888) reports having seen several wolves reared in captivity which had upright tails, and one wolf whose tail was turned to the right.

Three among our  $F_1$  hybrids had straight tails of the wolf type, but there is no information about the tails of the remaining  $F_1$  specimens. In  $F_2$  hybrids a definite segregation was observed: there were tails of the wolf type, slightly curved tails and sickle-shaped ones.<sup>2</sup>

The log-shaped tail on the one hand and the curved on the other may be considered to a certain degree as hereditary characters.

Hence the observations of Nehring, and of some other authorities, on wolves reared in captivity sometimes having curled tails may perhaps be explained as due to segregation rather than as a result of domestication as they suggest. Probably the differences between the dog and the wolf in the tail set are conditioned partly by modificatory and partly by genetical differences.

The question of tail *wagging* is rather interesting. According to the literature neither the wolf nor the jackal wag their tails, and this character might perhaps distinguish them from the dog.

Nevertheless, I affirm, that the wolf can learn to wag its tail. We have observed in the Moscow Zoopark wolves which have learned to wag theirs as dogs do. Mrs E. Iljin is of the opinion that every wolf can wag its tail even without preliminary training. This difference is of a purely phenotypical nature. It is connected with causal moments in wolf life. Taenzer (1923-5) reports that the jackal can also learn to wag its tail, and our own observations show that the fox also can wag its tail.

All our wolf-dogs are tail wagging.

#### (5) *The ear form (ear carriage)*

It is well known that the wolf has upstanding, erect ears with cartilaginous basis. Many races of dogs have drooping, lopping ears. German sheep-dogs, Laika dogs and a number of other races of dogs have erect, upstanding ears.

In the second generation of our wolf-dogs there was a definite segregation in the ear form, which could be readily explained if the

<sup>1</sup> However, Haacke mentions rare exceptions to this rule.

<sup>2</sup> The reader should not form conclusions as to the shape of the tails of our hybrids from our photos, because all our wolf-dogs drew in their tails out of fear of the photographic apparatus.

original dog had lopping ears, but on this point we have unfortunately no information.

Plate (1925, 1929), in dachshunds, found lop ear ( $H$ ) partially dominant to erect ( $h$ ).

My observations on fox terriers confirm this, for I have seen fox terriers with erect ears born of parents with lopping ears (Iljin, 1926, 1932).

Probably the inheritance of ear form is not limited by  $H$ ,  $h$  only. In certain races, e.g. collies, there is an obvious constancy in the inheritance of semi-erect ears, with upright lower three-quarters and a lopping tip. Evidently in this case the semi-erect ear is not due to  $H$  in the heterozygous state, but to the homozygous state of some other factor,  $H^a$  ("halb-hängend" or semi-erect). Collies crossed *inter se* usually produce specimens with such semi-erect ears. Nevertheless, I have several times observed in the progeny of  $H^a \times H^a$  collies segregation into semi-erect and lop. Evidently there exists a special gene  $H^a$ , which is dominant to  $H$ . We may assume the existence of a triple allelomorphic series:  $H^a$ ,  $H$  and  $h$ , where  $H^a$  conditions semi-erect ears of the collie type,  $H$  lop ears and  $h$  erect, upstanding ears.

$H^a H^a$  is phenotypically like  $H^a H$ , but  $Hh$  differs from  $HH$ , i.e. the semi-erect ear of the collie type ( $H^a$ ) is a completely dominant character, while the lop ear ( $H$ ) is an incompletely dominant one (see Iljin, 1932).

This scheme of the triple allelomorphs, probably applicable to dogs, may be applied in the case of our wolf-dog hybrids.

Among them are specimens with erect, upstanding, lopping semi-erect of the collie type, and semi-lop ears.

$H^a H^a$ ,  $H^a H$  and  $H^a h$  = semi-erect of the collie type.

$HH$  = lop.

$Hh$  = semi-lop.

$hh$  = erect.

Other factors probably enter into the inheritance of the ear form. Besides the types of ears mentioned there exists another, intermediate between the semi-erect of the collie type and semi-lop ears. In this type the lower half of the ear is hard and may be set upright (though usually lopping), while the upper half is lopped. I have several times observed such a type of ear in Russian borzoi, and Keller (1919) in Sudan wolf-hounds.

Again, certain dogs are characterized by another type of lop ear recessive to the erect type. Of the genetical nature of such types we are at present ignorant.



There would appear also to be some connexion between the ear lopping and ear length.

Modification of form with age also introduces complications. Upstanding, erect ears of Laika-dogs, of the German sheep-dogs and of wolves are lopping in puppies and acquire their typical form during the individual development of the animals (Iljin, 1937). This process is slower in insufficiently developed animals,<sup>1</sup> and the formation of erect ears may be considerably delayed for this reason. I hope to deal more fully with this subject in a future publication.

#### IV. INHERITANCE OF SKULL CHARACTERS

##### (1) *Material*

I had at my disposal twenty-nine hybrid skulls—viz. four  $F_1$  and twenty-five  $F_2$  specimens. Their study was facilitated by the fact that the  $F_2$  skulls belonged to the progeny of but two  $F_1$  pairs, i.e. fourteen to one pair, nine to the other. We also had two hybrid skulls whose origin was not completely established.

##### (2) *The difference between skulls of the dog and the wolf*

The old authorities were inclined to see a great number of differences between the skull of the wolf and that of the domestic dog. Thus Giebel (1859)<sup>2</sup> gave the following comparison of the dog's and the wolf's skulls:

Zwischenkiefer-, Stirn- und Scheitelkamm gleichen einander vollkommen, die Augenhöhlen des Wolfes sind etwas kleiner, die Jochbogen merklich niedriger und viel weniger aufwärts gekrümmt, der Vorderrand der Nasenbeine minder tief ausgebuchtet und die seitlichen Vorderecken nicht über den Intermaxillarrand vorspringend, die Nasenbeine selbst nach hinten viel schneller verschmälert merklich über den Frontalrand des Oberkiefers hinausreichend, der Scheitelkamm viel weiter nach hinten überragend die Hinterhauptsfläche in der oberen Hälfte merklich verschmälert, die Paukenknochen höher gewölbt, der Gaumen schmaler, die *foramina incisiva* ansehnlich länger und schmaler, der Winkelfortsatz des Unterkiefers stets breiter, stärker komprimiert und minder gekrümmt.

We see that Giebel points out a great many differences between the skull of the wolf and that of the dog, and their reality has been widely held up to the present. Nevertheless, the investigations of a number of authorities who worked at the end of the nineteenth century showed that: (1) there exist no qualitative but only quantitative differences,

<sup>1</sup> The ears may become lopping during the period of teething and in a few days become upright again.

<sup>2</sup> Giebel, C. G., *Die Säugetiere*, 1859 (cited from Taenzer, 1923-5).

and (2) the major number of these quantitative differences are indistinct, manifested in a number of intermediary transgressive forms.

Bogoljubsky (1928) describes the difference between the skull of the dog and that of the wolf in the following way:

Beim Wolfe ist der Schädel sehr gross, oder gross, selten von mittlerer Grösse, die Schnauze lang oder mittellang, die *Glabella* schwach ausgeprägt, das Stirndreieck kurz, die Schadel-Kapsel niedrig, die Schnauze flach, die Infraorbitalbrücke lang, selten mittellang, der Sagittalkamm hoch, seltener von mittlerer Höhe, die Grenzen des Gaumens vorn von  $M_2$  oder in der selben Fläche.

The author adds to the description a definition of the shape of the bullae tympani and "die Abwesenheit der Einbiegung der Basis cranii, die auch vorhanden sein kann, aber in diesem Falle sehr wenig ausgeprägt ist".

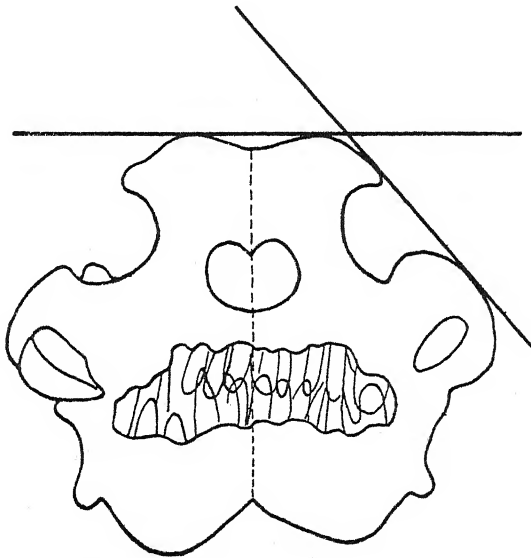
Bogoljubsky has shown (1928) that among wolves as well as among dogs there may be observed homological (according to Bogoljubsky, analogical) series in the manifestation of different skull characters. Hence the majority of the characters which were considered as typical for the taxonomic unit in question can by no means be regarded as characteristic, since they are met with both in wild and in domestic dogs.

Studer (1901), on the other hand, denied the importance of the characters stressed by earlier authorities (Giebel among them), regarding the position and the shape of the orbits as the only character in which domestic dogs invariably differ from the wild Canidae. The orbital axis, directed forward in dogs, is in wolves and jackals directed outwards and somewhat upwards. The orbital plane in dogs forms a more obtuse angle with the forehead plane. Hence the anterior outline of the dog's eye is more abruptly bent and the shape of its orbits rounder. The peculiar direction of the wolf's orbital plane leads to the oblique position of the eyes which gives it a less "noble" appearance than the dog.

The difference in the general type of the face of the wolf and the dog is due to the fact that the domestic dog has broader forehead cavities and, in general, a broader frontal part of the skull. The upper part of its skull roof in the region of the forehead is bent in comparison with the facial part of the skull. Therefore the forehead rises above the facial skull, the anterior margin of the eye is steeper, and the processus zygomaticus maxillae (pr. zyg. max.) juts out farther and somewhat backwards; the outlines of the orbit are rounder and the orbital plane is more steep. From above the orbits of the dog are less conspicuous than in the wolf or the jackal. Klatt (1913) supposes the latter fact to be connected with the size of the animal.

According to Studer the orbital angle alone affords a good criterion between the skull of the wolf and that of the dog.

The orbital angle is the angle formed by the orbital plane (drawn through the upper and the lower marginal edges of the eye) and the horizontal plane drawn through the upper margins of the ossa frontalia (Text-fig. 2). The orbital angle in wolves according to Studer is  $40-45^\circ$ , while in the majority of dog races it is  $53-60^\circ$ . Only primitive dogs, e.g. the deer-hound ( $52^\circ$ ), the German sheep-dog (two specimens,  $50^\circ$ ),



Text-fig. 2. Illustrating method of measuring the orbital angle (after Studer).

Catack-dog and *Canis palustris* (the stone-age dog) ( $48^\circ$ ), are somewhat nearer to the wolf, though they still differ from the latter.

Bockelmann (1920) confirmed Studer's results. He measured the orbital angle in four wolves. It ranged from  $41$  to  $42^\circ$ , while in dogs it was  $47-48^\circ$  and even  $56^\circ$ . He also found two skulls of primitive dogs, whose orbital angles were  $46$  and  $45^\circ$ . Bockelmann supposes the latter skulls to be those of natural dog-wolf hybrids (the so-called "Cajana-hund" from Finland). He also considers the orbital angle to be the only sure criterion in which the dog skull differs from that of the wolf.

Schäme (1922) considers the glabella to be the chief peculiarity of the skull of the domestic dog, thus confirming Studer, since the shape of the glabella is dependent upon the position of the orbits.

Nevertheless, the orbital angle is more reliable than the glabella shape because among the wolves as well as among the dogs occur parallel manifestations of different forms of glabella.

My own data confirm those of Studer and Bockelmann.

The orbital angles of four wolf skulls measured by me were 39.5, 41, 42 and 46.5°; of four German sheep-dogs, 52, 53, 54, 55°. Table 10 summarizes the results obtained by Studer, Bockelmann and myself.

Table 10. *The orbital angle in wolves and German sheep-dogs*

Student	Wolves	German sheep-dogs
Studer	40-45	50, 50
Bockelmann	41, 41, 42, 42	49, 50, 52
Iljin	39.5, 41, 42, 46.5	52, 53, 53, 54, 55
Limits of variations	39.5-46.5	49-55

The second character in which the skull of the wolf differs from that of the dog is the shape and the length of the processus zygomaticus ossis maxillaris (proc. zygom. max.).

Schäme (1922) found this process to be short in domestic dogs, its angle obtuse or almost right, and its end reaching the line of the medial sulcus of the first true molar tooth, while in wolves it is much longer (about 3-6 mm. long if measured from the point just above  $M_1$ ) and forms an acute angle.

My observations on the whole confirm those of Schäme (see later). The character is a convenient one for skull analysis.

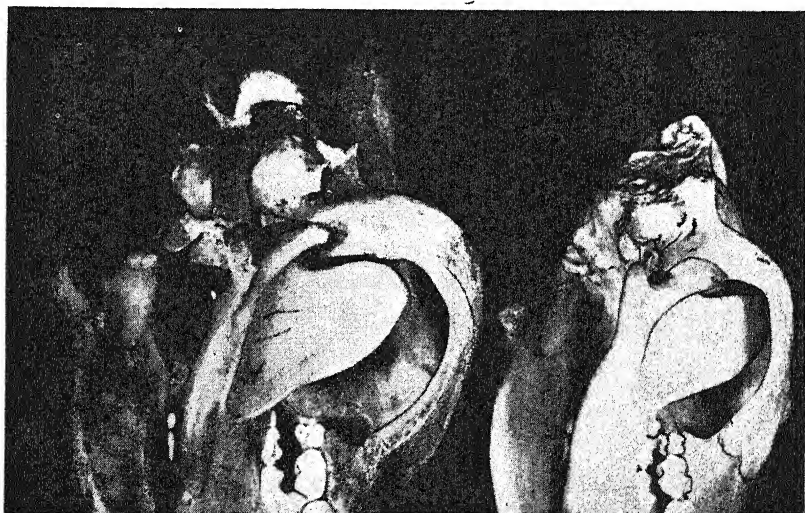
A third differentiating character (cf. Giebel) is the shape and size of the bullae tympani. In wolves they are large, convex, almost spherically shaped, without any ribs. In domestic dogs they are medium-sized or even very small, slightly convex, not roundish, strongly compressed or at any rate slightly crumpled with slightly or even strongly marked ribs (Text-fig. 3). Brauner (1928) reported them to be sometimes convex in certain specimens of south Russian sheep-dogs, but in this case they are of a small size. The only exception in dogs is the borzoi.

A fourth differentiating character, *size of skull*. This may be expressed by *basilar length*, i.e. the distance between the *gnathion* (the anterior margin of the praemaxillare at the *fissura* between the middle incisors on their outside), and the frontal lower margin of the foraminis occipitalis magni.

The basilar length of the wolf's skull ranges from 186 to 243 mm. according to Studer (1901), from 172 to 236 mm. according to Hilzheimer (1909), from 189 to 226 mm. according to Schäme. A huge 272 mm. long wolf skull was described by Nehring (cited from Studer). Brauner

(1928) has given the figure 218 mm. as the average of twenty-seven measurements. He says that only in young wolves was it less than 210 mm.

The basilar length in sheep-dogs ranges from 164 to 179 mm. according to Studer, from 164 to 193 mm. according to Schäme, that of collies from 170 to 192 mm. according to Marchlewski (1926). We may assume the length of the skull as 164–179 mm., because the majority of sheep-dogs (German in particular) are characterized by the above dimensions of the skull.



Text-fig. 3. Bullae tympani of a wolf (left) and of a dog (right).

Nevertheless, the value is diminished by the existence of parallel series in size of skull among wolves and sheep-dogs. Bogoljubsky (1928) distinguishes three groups of wolves: those with medium-sized skulls (basilar length 160–200 mm.), with large skulls (basilar length 200–230 mm.), and with huge skulls (basilar length over 230 mm.). Among sheep-dogs may be distinguished the two former groups only.

For this reason basilar length is an unsatisfactory character for our work, and it has a further disadvantage in varying greatly with age.

A fifth differentiating character is the *volume of the cranial capsule*. According to Klatt (1913) the volume of the wolf's skull is 150–170 cm.<sup>3</sup>, while that of the dog's skull is 120 cm.<sup>3</sup>

The *border line of the palate* in relation to the position of the second

molar tooth in the upper jaw may be considered as a sixth differentiating character of difference. According to Bogoljubsky the back margin of the palate in wolves is in front of  $M_2$  or in their plane, while in sheep-dogs it is behind  $M_2$ . The margin line of the palate is nevertheless a character which easily changes under the influence of external factors, as shown by the investigations of Wolfgramm (1894) who believes it to be connected with modificatory variations of the length of the muzzle.

We may take it that the skulls of wolves differ from those of dogs in the following six principal characters:

- (1) Orbital angle, the size.
- (2) Processus zygomaticus maxillae, shape and length.
- (3) Bullae tympani, shape and size.
- (4) The size of the skull, its basilar length in particular.
- (5) The volume of the cranial capsule.
- (6) The border line of the palate.

The above characters are those in which the skull of the wolf really differs from that of the dog. Other suggested characters either form parallel series in both animals or vary transgressively from one form to another, and could give reliable results only through a statistical investigation of a very great number of skulls. Therefore these characters are inconvenient for comparison.

### (3) *The causes of these differences*

The Lamarckist conceptions which have prevailed among many a student about the origin of the domestic dog have led them to suppose the differences between the dog and the wolf skulls to be purely modificatory, but many authors, e.g. Wolfgramm (1894), Keller (1919), Schäme (1924) and even to a certain degree Antonius (1922), probably overestimate the influence of the external factors upon the peculiarities of the skull structure.

How far captivity, through changes in feeding, muscular activity, etc., affects the skulls of the wolves and jackals we may now go on to consider.

Nehring (1884) reports a number of changes in the skull of the wolves kept in captivity, affecting the length and proportions of the skull, the length, shape and position of teeth. His data show that the length of the dens sectorius peculiar for wolves as exceeding the length of the two molars is a purely modificatory character in which the wolf differs from the domestic dog, this length of the dens sectorius in the latter is assumed as not exceeding the length of  $M_1 + M_2$ .

Wolfgramm (1894) has observed the following modifications in the skulls of wolves kept in captivity: an absolute decrease of all skull dimensions, a relative decrease of the length of the visceral part, a relative increase of the length of the neural part, a relative increase of the breadth of both parts, a relative increase in skull height. Thus the skulls of the wolves reared in captivity are shorter, broader and higher than those of wild wolves. Later on Wolfgramm pointed out the presence of modifications in the teeth system (in fangs, in *dentes sectorii*, somewhat less marked in molars), a "straightening" of the muzzle in connexion with the changes in the direction of the skull axes, the receding of the palate margin, the modifications in the position of the base of the cranial part of the skull, increase of the cerebral capsule, curvation of the frontal wall of the cranial part of the skull, etc. Wolfgramm considers these modifications as connected with the change in the conditions of life and nutrition, e.g. infrequent use of the fangs and reduction in the activity of the muscles.

Noak (1907) also observed a number of considerable modifications in the skulls of wolves and jackals reared in captivity. Their skulls are nearer to those of domestic dogs than are the skulls of wild wolves.

Klatt (1921) points out that the lack of muscular exercise in wolves and jackals kept in captivity inhibits the development of the parieto-occipital crest, and induces a widening of the parieto-temporal region. The skull becomes shorter and broader in accordance with Wolfgramm's data. Klatt is also inclined to connect the presence of great differences between the skulls of different dogs with the difference in the size of their body, but this explanation cannot be considered as sufficient for a number of differences.

Antonius (1922) on the whole confirmed Wolfgramm's data. Moreover, in a single case he described a wolf reared in captivity whose skull was long and narrow like that of the borzoi (Russian wolf-hound). Antonius is inclined to consider this phenomenon as a reaction of the external factors in the direction opposite to that observed by Wolfgramm (shortening of the frontal part and widening of the skull). Personally I consider genetic segregation of the long and narrow skull to be a more simple explanation of the phenomenon.

Schäme (1924) partly confirms the data of Wolfgramm and comes to the conclusion that the variations of glabella depend upon purely mechanical external factors, i.e. upon the wild canids mostly using their incisors and fangs, and domestic dogs their molar teeth.

It is clear that a number of characters of wolf, jackal and dog's

skulls can become modified under the influence of external conditions. Nevertheless, the differences between the dog's and the wolf's skulls cannot be explained as the result of the influence of external conditions only. We have no doubt that besides purely modificatory variations there are also hereditary differences.

Generally speaking the skulls of the wolves are subject to rather extensive individual variations notwithstanding their apparent homogeneity. I am not aware of mass biometrical investigations being reported in the literature, but the data quoted by a number of authorities perfectly confirm the above statement. Long ago Nehring (1884, 1890) and more recently Brauner (1928) pointed out considerable individual variations in all measurements of wolf skulls. The results of my own observations confirm this. Further, Wallace (1889), on the basis of J. A. Allen's work (1876), established a great variability of the skulls of the wolves which inhabit the same region of North America. Studer (1901) points out that the skull of the wolf is the most variable among the skulls of all the wild mammals. He also points out a great variability of the skulls which come from the same region and under similar conditions. Moreover, the authorities cited at the beginning of this section pointed out the *different* degrees of the skull modifications in *similar* external conditions.

Perhaps these materials indicate that the causes of variations in certain skull characters are of a genotypical nature. The existence of homological series in the wolf and dog skull characters suggests the same conclusion.

We consider as very important the investigation of the following questions: (1) how the skull characters in the dog and the wolf are inherited, and (2) whether the differences between the skull of the wolf and that of the dog are hereditary or not.

#### (4) *Segregation in skull characters*

For this study I have chosen characters which are the most marked and reliable points of the distinction between the skull of the wolf and that of the dog, namely,

- (1) Orbital angle.
- (2) The shape of the processi zygomatici maxillae (pr. zyg. max.).
- (3) The shape and the size of the bullae tympani.

Our wolf-dogs were kept under approximately the same conditions, which allows us to compare their skulls. Of course neither keeping nor



feeding were absolutely identical because of the competition between the puppies at feeding.

Schäme demonstrated (1922) that the final shape of the skull bones becomes established only towards the second juvenile stage (between 6–7–12 months). Therefore we never used for our studies skulls younger than that stage in order to avoid the influence of the age variations.

The material we worked on is summarized in Table 22 and single characters are dealt with in the following sections.

(a) *Orbital angle.*

The angles in each skull were measured seven or eight times. The first two measurements were not taken into account, but the average was calculated from the last five to six measurements.

The measurements of the orbital angles in the skulls of our wolf-dogs  $F_1$  and  $F_2$ , wolves and German sheep-dogs are summarized in Tables 11 and 12.

Table 11. *Orbital angle in wolves, German sheep-dogs and wolf-dogs*

	39	40	41	42	46	47	48	49	50	51	52	53	54	55	56	57	<i>n</i>
I. Wolves	1	—	1	1	1	—	—	—	—	—	—	—	—	—	—	—	4
II. German sheep-dogs	—	—	—	—	—	—	—	—	—	—	1	2	1	1	—	—	5
III. $F_1$	—	—	—	—	—	—	—	1	—	1	1	—	—	—	—	—	3
IV. $F_2$ ("Mamsel" × "Skukum")	—	—	—	—	1	1	1	3	3	1	3	1	—	—	—	—	14
V. $F_2$ ("Chernyshy" <i>inter se</i> )	—	1	—	—	—	1	3	2	2	—	—	—	—	—	—	—	9
VI. All the $F_2$ skulls: IV + V + $F_2$ skulls of unknown parents	—	1	—	—	1	2	6	5	5	1	3	1	—	—	—	—	25

$F_1$  animals have the orbital angle intermediate between the wolf and the sheep-dog, but a little nearer to that of the latter.

This does not agree completely with Bockelmann's data (1920); his  $F_1$  wolf-dogs approached the wolf, though the skull of one of them had the orbital angle of the dog type. Bockelmann mentions that his five  $F_2$  hybrids came from several pairs of animals. Therefore no definite conclusions can be formed on the basis of his data. The  $F_2$  hybrids of Bockelmann—four in number—gave the same figures as  $F_1$ .

In the second generation of our hybrids there is a distinct segregation which may be observed even in the progeny of a single pair of parents (Table 11, line IV or V).

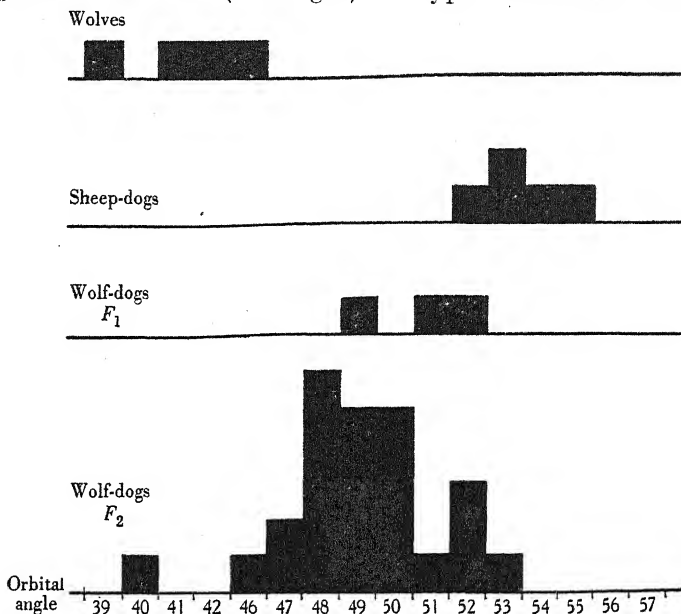
The examination of the total series of  $F_2$  skulls (line VI) makes the presence of this segregation still more evident. In  $F_2$  there segregate out

Table 12. *Principal skull characters of our wolves, German sheep-dogs and wolf-dogs*

No.	The no. or name	Orbital angle	Pr. zyg. max.		Bullae tympani		
			Right	Left	Size	Shape	Ribs
Wolves							
1	1	39	ac.	ac.	very large	convex	none
2	2	46.5	ac.	ac.	very large	convex	none
3	3	42	ac.	ac.	very large	convex	none
4	4	41	ac.	ac.	large	convex	none
German sheep-dogs							
5	"Irlando" (male)	55	opened	opened	minute	flat	ribs
6	"Treff" (male)	53	opened	opened	—	—	—
7	"Anita" (female)	54	opened	opened	middle-sized	rumpled	ribs
8	23 (male)	52	opened	opened	small	flattened	edging
9	24 (male)	53	opened	opened	small	slightly rumpled	hints
10	"Zanda" (female)	—	—	—	middle-sized	rumpled	slight
Wolf-dogs $F_1$							
11	1	49.1	int.	int.	large	slightly rumpled	hints
12	2	51	int.	int.	middle-sized	—	—
13	3	51.75	obt.	obt.	middle-sized	slightly rumpled	hints
14	4	—	int.	obt.	large	slightly rumpled	slight
Wolf-dogs $F_2$							
Progeny from (no. 8 "Mamsel" × no. 7 "Skukum")							
15	2	48	ac.r.	ac.r.	large	slightly rumpled	hints
16	3	51.75	ac.	ac.	middle-sized	flattened	slight
17	4	52.5	ac.	ac.	small	flattened	slight
18	5	49	ac.	ac.r.	middle-sized	rumpled	slight
19	6	51	ac.	ac.	middle-sized	rumpled	slight
20	3a	49	ac.	ac.	middle-sized	rumpled	slight
21	5a	49.25	ac.	ac.	middle-sized	rumpled (left is flattened)	slight
22	7a	50.5	arch.	arch.	small	flattened	slight
23	8a	53	ac.	ac.r.	middle-sized	flattened	slight
24	10	50.5	ac.r.	ac.r.	middle-sized	rumpled	middle
25	11	50.5	ac.	ac.	middle-sized	rumpled	edging
26	12	47.5	ac.r.	ac.r.	middle-sized	rumpled	slight
27	13	52.5	ac.r.	arch.	middle-sized	rumpled	slight
28	14	46.4	ac.	ac.r.	middle-sized	flattened	edging
Wolf-dogs $F_2$							
Progeny from ("Chernyshy" <i>inter se</i> : no. 10 × no. 9)							
29	1	47	int.	ac.r.	very large	convex	left none—right hints
30	7	48	ac.	ac.r.	large	rumpled	slight
31	8	50	int.	int.	middle-sized	flattened	slight
32	9	49.5	int.	obt.	small	flattened	strongly ribbed
33	15	49.5	ac.r.	ac.	small (right minute)	flattened	strongly ribbed
34	1a	49.9	ac.	ac	large	left slightly flattened — right slightly convex	left slight—right hints
35	2a	48	ac.	ac.	middle-sized	slightly rumpled	slight
36	4a	48.5	ac.	ac.r.	small (minute)	flattened	edging
37	6a	40.5	ac.r.	ac.	middle-sized	rumpled	slight
Wolf-dogs $F_2$							
(Parents not precisely known)							
	17	48.5	ac.	ac.	large	rumpled	hints
	16	48	ac.	int.	large	slightly rumpled	hints

typical wolf skulls ( $40.5^\circ$  angle), typical dog skulls ( $52-53^\circ$  angle) and intermediate skulls ( $47-51^\circ$  angle). The latter are the most numerous.

We have uniformity in the first hybrid generation and evident segregation in the second (Text-fig. 4). The type of inheritance is evidently

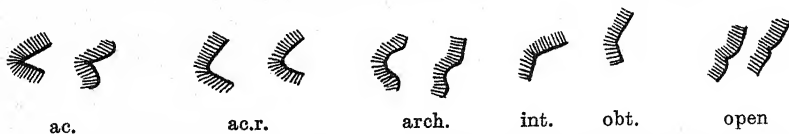


Text-fig. 4. Size inheritance of the orbital angle in wolf-dog crosses. Abscissae = size of orbital angle. Ordinates = number of skulls.

conditioned by polymeric factors. The frequency of the extreme figures ( $41-53^\circ$ ) leads us to suppose the number of genes concerned to be not more than two.

(b) *The shape of the processi zygomatici maxillae. Its frequent asymmetry.*

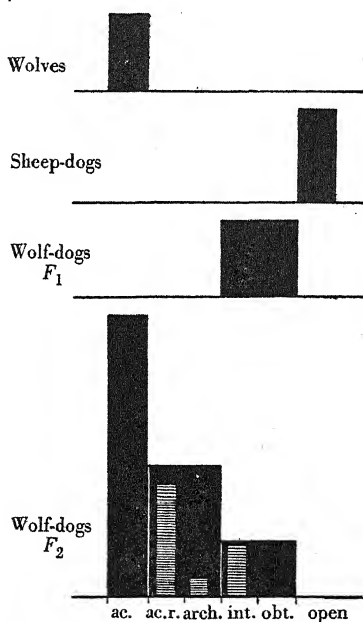
The study of this character on the wolf-dog skulls allowed us to establish five or even six types of the shape of the pr. zyg. max. The angle at which it projects from the zygomaticus bone may be characterized as follows: acute (ac.), acute with a somewhat rounded point (ac.r.), arched (arch.), intermediate (int.), obtuse (obt.), opened almost up to  $180^\circ$  (op.). All the above types are schematically represented in Text-fig. 5.



Text-fig. 5. Types of the shape of the pr. zyg. max. in wolves, dogs and their hybrids.

Several skulls have asymmetric angles of the pr. zyg. max. In our case there were 56 % asymmetric skulls in  $F_2$  (14 out of 25). In 82 % of all the asymmetric skulls the right angle is nearer to the wolf type than the left one (Table 13). The same phenomenon may be observed in wolves and German sheep-dogs. This fact is rather interesting, though its causes are as yet unknown.

The skulls of the wolves I examined had a typically acute angle, while those of German sheep-dogs possessed a typically open one (Pl. 24, figs. 15, 16, Table 14).



Text-fig. 6. Pr. zyg. max. shape inheritance in wolf-dog crosses.

Our  $F_1$  hybrids (Table 15) had intermediate or obtuse angles (Pl. 25, figs. 17, 18). In the second generation can be noticed an obvious segregation (Pl. 26, figs. 19, 20 and Tables 16, 17, 18). Nevertheless, despite a relatively great number of skulls we have not met with typically dog angles of the pr. zyg. max. in the second generation (Text-fig. 6). There were intermediate skulls (like those of the  $F_1$ , intermediate and obtuse), skulls which approximate the wolf type (arch.) and typical wolf skulls (ac. and ac.r.), but none of the twenty-five skulls had typical dog angles (open up to  $180^\circ$ ), while eleven or fifteen had angles of the wolf type. Only one skull (No. 9) could have been classified as intermediate between the acute and open angles.

No. of skulls	The angle on right side	The angle on left side	The side which is nearer the wolf type
Wolf	ac.	ac.	right
German sheep-dogs:			
"Irlando"	op.	op.	right
"Tref"	op.	op.	right
Hybrids			
4	ac.	ac.	right
5	ac.	ac.r.	right
7 <i>a</i>	arch.	arch.	right
8 <i>a</i>	ac.	ac.r.	right
11	ac.	ac.	right
13	ac.r.	arch.	right
14	ac.	ac.r.	right
1	int.	ac.r.	—
7	ac.	ac.r.	right
8	int.	int.	right
9	int.	obt.	right
15	ac.r.	ac.	—
4 <i>a</i>	ac.	ac.r.	right
6 <i>a</i>	ac.r.	ac.	—
			14
			3

No. and name	Age	Angle on the right side					Angle on the left side						
		ac.	ac.r.	arch.	int.	obt.	op.	ac.	ac.r.	arch.	int.	obt.	op.
Wolves													
Wolf 1	Adult	+	—	—	—	—	—	+	—	—	—	—	—
Wolf 2	Adult	+	—	—	—	—	—	+	—	—	—	—	—
Wolf 3	Adult	+	—	—	—	—	—	+	—	—	—	—	—
Wolf 4	Adult	+	—	—	—	—	—	+	—	—	—	—	—
German sheep-dogs													
Sheep-dogs:													
♂ "Irlando" 9 m.		—	—	—	—	—	+	—	—	—	—	—	+
♂ "Tref"		—	—	—	—	—	+	—	—	—	—	—	+
♀ "Anita" About 5 yr.		—	—	—	—	—	+	—	—	—	—	—	+
♂ 23 3 yr.		—	—	—	—	—	+	—	—	—	—	—	+
♂ 24 4 yr.		—	—	—	—	—	+	—	—	—	—	—	+

No. of the skulls	Age in months	The angle on the right side						The angle on the left side					
		ac.	ac.r.	arch.	int.	obt.	op.	ac.	ac.r.	arch.	int.	obt.	op.
1	About 16	—	—	—	+	—	—	—	—	—	+	—	—
2	"	—	—	—	+	—	—	—	—	—	+	—	—
3	"	—	—	—	—	+	—	—	—	—	—	+	—
4	"	—	—	—	+	—	—	—	—	—	—	+	—
		—	—	—	3	1	—	—	—	—	2	2	—

Table 16. *The shape of the pr. zyg. max. of the  $F_2$  hybrids of dog and wolf. (Progeny from ♀ "Mamsel" × ♂ "Skukum")*

No. of the skulls	Age in months	The angle on the right side						The angle on the left side					
		ac.	ac.r.	arch.	int.	obt.	op.	ac.	ac.r.	arch.	int.	obt.	op.
2	6	—	+	—	—	—	—	—	+	—	—	—	—
3	6	+	—	—	—	—	—	+	—	—	—	—	—
4	6	+	—	—	—	—	—	+	—	—	—	—	—
5	6	+	—	—	—	—	—	—	+	—	—	—	—
6	6	+	—	—	—	—	—	+	—	—	—	—	—
3a	More than 6	+	—	—	—	—	—	+	—	—	—	—	—
5a	More than 6	+	—	—	—	—	—	+	—	—	—	—	—
7a	More than 6	—	—	+	—	—	—	—	—	+	—	—	—
8a	More than 6	+	—	—	—	—	—	—	+	—	—	—	—
10	21	—	+	—	—	—	—	—	+	—	—	—	—
11	21	+	—	—	—	—	—	+	—	—	—	—	—
12	21	—	+	—	—	—	—	—	+	—	—	—	—
13	21	—	+	—	—	—	—	—	—	+	—	—	—
14	21	+	—	—	—	—	—	—	+	—	—	—	—
Total		9	4	1	—	—	—	6	6	2	—	—	—
17		+	—	—	—	—	—	+	—	—	—	—	—
16*		+	—	—	—	—	—	—	—	—	+	—	—

\* Two skulls  $F_2$  from unknown parents.Table 17. *The shape of the pr. zyg. max. in  $F_2$  hybrids of dog and wolf. (Progeny from "Chernysky" inter se)*

No. of the skulls	Age in months	The angle on the right side						The angle on the left side					
		ac.	ac.r.	arch.	int.	obt.	op.	ac.	ac.r.	arch.	int.	obt.	op.
1	6	—	—	—	+	—	—	—	+	—	—	—	—
7	6	+	—	—	—	—	—	—	+	—	—	—	—
8	6	—	—	—	+	—	—	—	—	—	+	—	—
9	6	—	—	—	+	—	—	—	—	—	—	+	—
15	6	—	+	—	—	—	—	+	—	—	—	—	—
1a	6	+	—	—	—	—	—	+	—	—	—	—	—
2a	6	+	—	—	—	—	—	+	—	—	—	—	—
4a	6	+	—	—	—	—	—	—	+	—	—	—	—
6a	6	—	+	—	—	—	—	+	—	—	—	—	—
Total		4	2	—	3	—	—	4	3	—	1	1	—

Table 18. *The shape of the pr. zyg. max. of wolves, German sheep-dogs and  $F_1$  and  $F_2$  wolf-dog hybrids. (Summary of Tables 14, 15, 16 and 17)*

	The angle on the right side						The angle on the left side					
	ac.	ac.r.	arch.	int.	obt.	op.	ac.	ac.r.	arch.	int.	obt.	op.
Wolves	4	—	—	—	—	—	4	—	—	—	—	—
German sheep-dogs	—	—	—	—	—	5	—	—	—	—	—	5
$F_1$	—	—	—	3	1	—	—	—	—	2	2	—
$F_2$	15	6	1	3	—	—	11	9	2	2	1	—
Total in $F_2$	15	7		3		—	11	11		3		—
Expected 9 : 3 : 3 : 1	14.04	4.68		4.68		1.56	14.04	4.68		4.68		1.56

This apparent absence of segregation of the pure dog type may be explained in one of the following ways:

(1) The difference in the angles of the pr. zyg. max. of the wolf or the dog is not hereditary.

(a) The shape of the angle is a modificatory character. In  $F_1$  and  $F_2$  we find individual variability in the shape of the pr. zyg. max. superioris. (Schäme (1922) points out the importance of taking into account the juvenile stage of the angle. In our case the possibility of the juvenile stage of the angle is unconditionally excluded.)

(2) The shape of the angle is an hereditary character.

(b) Segregation is practically absent, since the wolf has no allelomorphs for the genes of the dog-type angle of the pr. zyg. max. In this case the variation in the shape of the pr. zyg. max. in  $F_2$  should be considered as a manifestation of individual variability.

Table 19. *Observed and expected segregation figures in the shape of the pr. zyg. max.*

	ac.	ac.r. and arch.	int. and obt.	opened	$\Sigma$
Expected 9 : 3 : 3 : 1	14.04	4.68	4.68	1.56	25
Observed on the right	15	7	3	—	25
Difference	+0.96	+2.32	-1.68	-1.56	—
Observed on the left	11	11	3	—	25
Difference	-3.04	+6.32	-1.68	-1.56	—

(c) Segregation has taken place. Different shapes of the pr. zyg. max. are the manifestation of different genotypes. An angle of the pure dog type did not segregate out because of a relatively small number of progeny ( $n=25$ ). This hypothesis may be true only under the condition of the inheritance of the wolf shape of the angle being dependent upon at least two dominant genes, and that of the dog type upon two recessive ones. This explanation does not agree well with polymeric inheritance because the segregation curve in  $F_2$  is "one-branched" (purely wolf angles on the right, 15; acute rounded, 6; arched, 1; intermediary, 3).

The first explanation is inadmissible because in this case (with hybrids kept in captivity) we should expect preponderance of angles of the dog type, whereas we actually obtained twenty-one skulls of the wolf or similar type out of twenty-five hybrid skulls.

Of the two other explanations I consider the third as the most probable one. Indeed, the figures obtained agree well even with the elementary segregation 9 : 3 : 3 : 1 (Table 19).

Hence we may draw the conclusion that the shape of the angle of the pr. zyg. max. may be inherited through two pairs of independent

genes with a complete or almost complete dominance, those of the wolf type being dominant.

It should be mentioned that the acute shape of the angle of the pr. zyg. max. is proper to certain primitive dogs with upright ears, the so-called Laika-dogs of northern regions of the U.S.S.R. The shape of these angles is almost identical with that of the wolf type. I noticed it in almost all the skulls of Laika-dogs which I was able to examine: in four skulls from the Komi region, nine skulls of the "Vogul" Laika-dogs, etc., and others.

I believe this to be a proof of Laika-dogs being continually crossed with wolves and being "saturated" with wolves' genes. Reports of Laikas crossing with wolves have been received from native owners.

(c) *The shape and the size of the bullae tympani.*

The difference between the dog and the wolf in the shape and the size of the bullae tympani is a very characteristic one (Text-fig. 3). Observations on a great number of skulls have shown that this character may be conveniently used in order to distinguish the skull of the wolf from that of the dog.

It is true that Wolfgramm has recorded some changes in the shape of the bullae in wolves kept in captivity; nevertheless, the *type* of the bullae remained unchanged. Since all our hybrids were kept in captivity, this fact may of course involve some correction in our judgement as to the shape of the bullae, but we may compare the latter among themselves.

My observations have shown the following differences in our wolf-dogs:

- (1) In the comparative size: very large—large—middle-sized—small—minute.
- (2) In the shape rotundity: convex—spherically rounded—slightly convex—rumpled—flattened out (compressed).
- (3) In the ribbedness: no ribs—hints of ribs—slightly ribbed—strongly ribbed at the edges.

These characters may be manifested independently.

In wolves the bullae are large, spherically rounded without ribs, whereas in German sheep-dogs they are small or minute, flat with ribs (Text-fig. 3 and Pl. 27, fig. 21).

Our hybrids  $F_1$  (Tables 20–22) have large bullae, almost as large as those of wolves, slightly flattened (Pl. 27, fig. 21).

In the second generation (Tables 20–22) there is evident segre-



Table 20. *The shape of the bullae tympani in wolves, German sheep-dogs and wolf-dog hybrids,  $F_1$  and  $F_2$* 

	Convex spherically rounded	Slightly convex	Slightly rumpled	Rumpled	Flattened	Total
I. Wolves	4	—	—	—	—	4
II. German sheep-dogs	—	—	1	2	2	5
III. $F_1$	—	—	3	—	—	3
IV. $F_2$ ("Mamsel" × "Skukum")	—	—	1	8	5	14
V. $F_2$ ("Chernyshy" <i>inter se</i> )	1	1*	1 + (1)†	2	4	9
VI. All the $F_2$ skulls (IV + V + two skulls of unknown parents)	1	1	3 + (1)	11	9	25

\* On one side.

† On one side, on the other slightly convex.

Table 21. *Size of the bullae tympani in wolves, German sheep-dogs and wolf-dogs,  $F_1$  and  $F_2$* 

	Very large	Large	Middle- sized	Small	Minute	Total
I. Wolves	3	1	—	—	—	4
II. German sheep-dogs	—	—	2	2	1	5
III. $F_1$	—	2	2	—	—	4
IV. $F_2$ ("Mamsel" × "Skukum")	—	1	11	2	—	14
V. $F_2$ ("Chernyshy" <i>inter se</i> )	1	2	3	3*	(2)†	9
VI. All $F_2$ skulls (IV + V + two skulls of unknown parents)	1	5	14	5	(2)	25

\* Two skulls with a small bulla on one side.

† On one side, on other small bullae.

Table 22. *The ribbedness of the bullae tympani in wolves, German sheep-dogs and wolf-dogs  $F_1$  and  $F_2$* 

	No ribs	Hint of ribs	Slightly ribbed	Ribbed	Edging	Total
I. Wolves	4	—	—	—	—	4
II. German sheep-dogs	—	1	3	—	1	5
III. $F_1$	—	2	1	—	—	3
IV. $F_2$ ("Mamsel" × "Skukum")	—	1	10	1	2	14
V. $F_2$ ("Chernyshy" <i>inter se</i> )	1*	1†	4	2	1	9
VI. All $F_2$ skulls (IV + V + two skulls of unknown parents)	1	4	14	3	3	25

\* On one side, on the other side traces.

† On one side, on the other slightly ribbed.

gation. The majority of  $F_2$  have an intermediary type of bulla, several skulls are nearer the dog type, one or two skulls have typical dog bullae and one has bullae of the wolf type (Pl. 28, fig. 22).

Hence the shape and the size of the bullae are evidently inherited according to Mendelian laws.

*The larger size of the bullae of the wolf is almost completely dominant over the smaller size of those of the dog. In respect of rotundity and flatness  $F_1$  is intermediate but nearer to the dog type. For absence of ribs in  $F_1$  is also intermediate. In  $F_2$  there is evident segregation in all the above characters.*

(d) *Other skull characters. Independence in the inheritance.*

Besides the orbital angles, the shape of the pr. zyg. max., the size, rotundity and ribbedness of ear bladders, the hybrid skulls show an evident segregation in a number of other characters. Even a superficial examination of  $F_2$  shows heterogeneity in the following characters: the cheek-bone breadth, the total size of the skull, the degree of the curvature in glabella (slight differences), and the dimensions of the sagittal crest.

The cheek-bone breadth is worth separate mention. According to Brauner (1928) this is 58.7–65.7% of the basilar length in wolves. According to Bogoljubsky there are three groups of wolves narrow-cheeked (below 60%), intermediate (60–63%), broad-cheeked (over 63%).<sup>1</sup> Sheep-dogs are narrow-cheeked (below 60%) (Pl. 29, fig. 24). Pl. 29, fig. 23 represents two skulls of our wolves: a very broad skull and an intermediate one.

Broad-cheeked skulls observed in our case as a result of segregation are probably the skulls of the wolf type. Broad-cheeked skulls are met with also in dogs, e.g. in Caucasian sheep-dogs with an index of about 63.7% (Brauner), and this of course tells against our suggestion. Nevertheless, the segregation in the cheek breadth in  $F_2$  is obvious, while the  $F_1$  hybrids are characterized by an intermediate cheek breadth (Pl. 29, figs. 25, 26).

Schäme has recently studied the skull breadth of the Canidae. He measured the upper-jaw breadth and the basal breadth in relation to the length of the upper jaw, to that of zygomatic arch and to the basilar length. He came to the conclusion that among all races of domestic dogs and wolves there are two chief forms: Leptocephalic and Plato-

<sup>1</sup> Bobrinsky (1928) points out the presence of wolves with narrow cheek bones as a taxonomic unit, the Saratov wolf (U.S.S.R.) *Canis lupus* subsp.

cephalic. The first group is characterized by a narrow skull with long jaws, the second by a broad skull with short jaws. Both forms are met with in four main variations: dwarf, small, intermediate ("normal") and giant.

The study of a few skulls (four skulls of  $F_1$  and one skull of  $F_2$  hybrids of different races of dogs and one skull of wolf-dog hybrid  $F_1$ ) led Schäme to suppose that the broad skull and narrow skull are hereditary characters, that the broad upper jaw is dominant to the narrow one and the greater basal breadth to the smaller one. In general the broad skull is dominant to the narrow one.

Our studies have led us to suppose the breadth of the skull to be an hereditary property in another character (the cheek-bone breadth). This is not ordinary monohybrid inheritance and dominance but rather a polymeric inheritance with intermediate  $F_1$  forms which are nearer to the greater skull breadth.

We may mention in conclusion that the examination of individual skulls does clearly prove *independence* in the manifestations of hereditary skull characters.

Thus,  $F_2$  skull no. 1 has bullae of the wolf type, intermediate angle of pr. zyg. max. and intermediate orbital angle ( $47^\circ$ ).

$F_2$  skull no. 8 has narrow cheek breadth (of the dog type?), intermediate angle of pr. zyg. max. and the orbital angle of the dog type ( $50^\circ$ ).

$F_2$  no. 4 and  $F_2$  no. 8a have the orbital angle of the dog type ( $52.5^\circ$ ) and the angle of pr. zyg. max. of the wolf type (acute).

$F_2$  no. 13 orbital angle of the dog type ( $52.5^\circ$ ) and the pr. zyg. max. acute, somewhat rounded, i.e. almost of the wolf type, etc.

These data clearly show independence in the manifestation of different hereditary characters of the skull. We may now make a suggestion as to the different allelomorphs for the skull characters studied (Table 23).

These are more or less definitely established hereditary characters. They may be supplemented with certain characters which Schäme studied on five  $F_1$  skulls and one  $F_2$  skull. He made some conjectures about the way in which they are inherited. Of course the study of  $F_1$  only does not allow of any conclusions as to the existence of special hereditary factors. Nevertheless, until further investigations are carried out we give here provisional symbols for these characters as a continuation of our list of genes:

- I. The greater breadth of the upper jaw is dominant to the smaller one ... ..  $H > h$
- II. The greater basal breadth is dominant to the smaller one  $I > i$

- III. The shortened parietalia are dominant to the elongated ones ... ..  $K > k$
- IV. The shortened upper jaw is dominant to (or is intermediary to) the elongated one ... ..  $L > l$
- V. The elongated zygomatic arch is dominant to the shortened one ... ..  $M > m$

Certain of these characters are probably reflexions of the same hereditary character. For instance, **H** and **I** are probably manifestations of our genes **G<sub>1</sub>** and **G<sub>2</sub>**, **K** and **L** of certain other genes, etc.

Table 23. *Suggested factors of the characters of the dog and wolf skulls*

No.	Character	Allelo-morph	Mode of inheritance	The type of $F_1$
1	Orbital angle	$A_1 > a_1$ $A_2 > a_2$	} Polymeric factors	Intermediate, nearer to German sheep-dogs
2	The shape of the angle of the pr. zyg. max.: acute—intermediate—open	$B > b$ $C > c$	} Independent factors with almost complete dominance	Intermediate, nearer to the wolf type
3	The size of the bullae tympani: large—intermediate—small—minute	$D_1 > d_1$ $D_2 > d_2$	} Polymeric factors (the number of pairs not precisely known)	Almost of the wolf type
4	Rotund—compressed bullae tympani	$E_1 > e_1$ $E_2 > e_2$	} Polymeric factors (the number of pairs not precisely known)	Intermediate
5	No ribs—ribs on the bullae tympani	$F_1 > f_1$ $F_2 > f_2$	} Polymeric (?) factors (the number of pairs not precisely known)	Intermediate, nearer to the sheep-dog type
6	The cheek-bone breadth	$G_1 > g_1$ $G_2 > g_2$	} Blending type of inheritance. Polymeric (?) factors (the number of pairs not precisely known)	Intermediate, nearer to the wolf type

Among other species little is known about the inheritance of cranio-logical characters. Mendelian segregation was established in rabbits by MacDowell (1914) and later on by Philipptschenko (1917), whereas Castle *et al.* (1909, 1916) supposed skull characters to be subject to blending inheritance without segregation in  $F_2$ .

Cranio-logical species differences were also noted by Detlefsen (1914) in the cross between *Cavia rufescens* and *C. porcellus*, though only for segregation in the sutura naso-frontalis character.

Here also should be mentioned Wriedt (1929), in connexion with length of muzzle and broad skull in offspring of Schnauzer-Dachshund by Pekingese.

## V. PHYSIOLOGICAL PECULIARITIES OF THE WOLF-DOGS

(1) *Fertility and sterility*

All our  $F_1$ ,  $F_2$  and  $F_3$  hybrids which had been mated proved capable of producing progeny.

A number of earlier authorities on the contrary stated that the capacity of wolf-dogs to produce young is limited to few generations (Flourence, 1855; Mivart, 1890, cited from Tracy, 1930; see also Wallace, 1889 and Darwin, 1868). Others reported that the fertility of wolf-dogs is low even at the beginning of cross-breeding (Zoological Garden in Hanover, Roerig, 1903).

Such low fertility was regarded as due to the interspecific cross itself, though Wallace believed it to be connected with the accumulation of bad peculiarities through inbreeding. This is not so, and in most cases such reports are the result of mistakes. Some authorities had reported still earlier a fertility of the wolf-dogs (Buffon, 1776; Broca *et al.* 1868; Bockelmann, 1920). The hybrids of the jackal and the domestic dog are also fertile when crossed *inter se* "even in closest parentage" (Kühn, 1887, cited from Lotsy, 1922).

I take the liberty to affirm that the wolf-dogs are absolutely fertile.

It is interesting that interspecific hybrids between dog and wolf are fertile when crossed with another closely related species. In this way Hiltzheimer succeeded in obtaining triple hybrids, wolf-jackal-domestic dogs, at the Zoological Garden at Halle (Lotsy, 1922).

(2) *Rut and pregnancy*

Wolves rut once a year (Brehm, Satunin): old wolves late in December and early in January, young ones late in January and early in February (according to Brehm). In Moscow wolves (according to Satunin) rut begins late in February, but the rivalry between the males and their chasing of the females begins at the end of December. At the Moscow Zoopark we have observed rut in wolves in January or February.

Dogs usually rut twice a year, in autumn or winter and in spring. Many Laika-dogs offer peculiar exception in that rut is exceptionally long and recurs several times a year. Some specimens among the small-sized races may rut three times a year.

Our hybrids as a rule rutted once a year, both in  $F_1$  and  $F_2$  generations. This is a manifestation of the wolf character in both the  $F_1$  and in the majority of  $F_2$  specimens. Our observations are incomplete, therefore I do not feel justified in making definite conclusions, though some  $F_2$

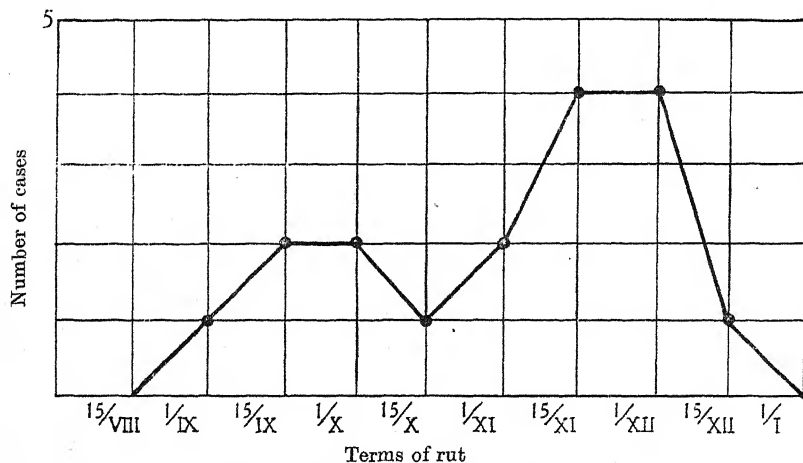
wolf-dog hybrids rutted twice (?) a year. If this observation is confirmed, there may be segregation of the dog type.

The rut terms are given in Table 24 and Text-fig. 7. The maximal number of ruts takes place between 1 November and 15 December.

According to Cuvier (see Darwin, 1868) the pregnancy in wolves lasts 2 months and several days; according to Saint-Hilaire 60–63 days; according to Wunderlich 62–66 days; to Heinroth (1908) 63 days; to Brehm 63–64 days; and to Satunin 63–65 days.

Table 24. *The rut terms in wolf-dogs*

	15. viii-l.	ix.-15.	ix.-1.	x.-15.	x.-1.	xi.-15.	xi.-1.	xii.-15.	xii.-1.	i.-15.	i. n
The number of cases	—	1	2	2	1	2	—	4	4	1	— 17



Text-fig. 7. Terms of rut in wolf-dogs.

Darwin states that pregnancy in big dogs lasts about 63 days, in small ones about 60–63 days. Our observations on the pregnancy of dogs in kennels give an average of 63 days with deviations of about 3–4 days, i.e. 60–66 days.

The pregnancy in our wolf-dogs of the  $F_1$ ,  $F_2$  and  $F_3$  generations lasted 60–62–63 days, like that in wolves and in dogs. Hunter in 1789 observed the pregnancy of a wolf-dog hybrid and found it to be equal to about 63 days (from Darwin).

The pregnancy in the jackal lasts 60–63 days according to Saint-Hilaire, and in a jackal-dog hybrid 59 days according to Hunter.

Hence there is practically no difference in the duration of the pregnancy in wolf, dog or jackal.

(3) *The litter size and the development of the young. Moulting*

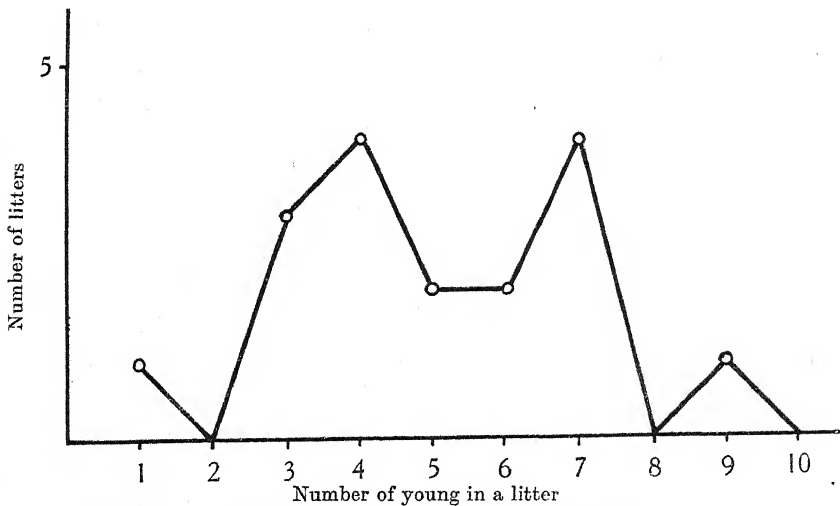
Brehm states that the number of young in a wolf litter ranges from three to nine, and for the most part four to six. Satunin (1895) gives the number four to eight for the Moscow wolves.

As far as I observed the maximal number of young wolves in a litter is twelve. Mrs E. Iljin found thirteen embryos in a bitch wolf.

The number of young for the dog varies a great deal, from one to

Table 25. *The litter size in wolf-dogs*

No. of young in a litter ...	1	2	3	4	5	6	7	8	9	10	No. of litters
No. of litters $F_1 \times F_1$	—	—	1	1	1	2	4	—	1	—	10
No. of litters $F_2 \times F_2$	1	—	2	3	1	—	—	—	—	—	7
$\Sigma$	1	—	3	4	2	2	4	—	1	—	17

Text-fig. 8. Litter size in  $F_1$  and  $F_2$  wolf-dogs.

fourteen to sixteen. I know of cases of twenty and even twenty-two puppies in a litter, though all of them were born dead.

For data on the litter size in the wolf-dog see Table 25 and Text-fig. 8.

At first sight  $F_2$  seems to give a smaller litter size than  $F_3$ , but this is probably fortuitous.

Young puppies of both the  $F_1$  and  $F_2$  hybrids begin to open their eyes on the 11th or 12th day. In one litter of  $F_1$  hybrids crossed *inter se* the puppies began to open their eyes on the 6th day after birth. A little later the puppies begin to move energetically, they try to crawl, and

learn to do so towards the 15th to 17th day; they begin to run towards the 24th to 27th day after birth.

According to our observations at the Moscow Zoopark wolf-puppies open their eyes also on the 11th to 12th day, the puppies of the German sheep-dog on the 9th to 12th day, rarely on the 14th day after birth.

The first teething and the change of the milk teeth in wolf-dogs are similar to those in dogs. The change of milk teeth seems to proceed at a somewhat higher rate. The first teeth begin to cut at the end of the 2nd or the beginning of the 3rd week and cease to cut at the 4th to 5th week.

The wolf-dogs moult twice a year like wolves. The spring moult takes place in March–April, lasting sometimes till May, rarely till June. The wolves kept in Moscow Zoopark also began to moult in April or early in May. The terms of the autumn moult were also the same in wolf-dogs and in dogs.

#### (4) *Barking and certain peculiarities of behaviour*

The wolf usually howls in different tones, and its howl is often long and dreary.

The capacity of barking proper to domestic dogs is not proper to any of the wild species, at least in the same form as in dogs.

Our  $F_1$ ,  $F_2$  and  $F_3$  hybrids bark very well.

Actually this habit may be acquired both by dogs which formerly could not bark, and by wild canids. Cuvier (1806), Darwin (1868) and Haacke (1903) report cases of wolves kept in captivity which learned to bark like dogs. Georgi (1800) affirms that polar foxes can bark and howl almost like dogs, etc.

I have observed at our laboratory “Giliat’s” Laika-dogs which at first could not bark, but who learned to in a very short time. One of my pupils, B. Lebedew (Smolensk), informed me that according to his observations a wolf can learn to bark in about a week. Saint-Hilaire (1863) possessed a jackal who could bark almost as well and in the same voice as a dog. I may mention that in the usual howl of wolves and jackals there may be sometimes heard sounds which remind one of barking. Mrs E. Iljin reports having observed a number of wolves who could bark very well (even among those which had just been caught). They barked when excited, as for instance at feeding, etc.

On the other hand Darwin reports a number of cases when dogs brought into solitary retired places (Guinea coast, Juan-Fernandez



island, Juan de Nova island, La Plata) forgot barking; their offspring howled instead of barking.

Thus we see that the difference between the wolf and the dog as to their capacity to bark is not an essential one, because both can learn and unlearn to bark. Therefore the report of Roerig (1903), who observed at the Marseilles Zoo a wolf-dog hybrid which "barked like a dog and had the exterior of a wolf", loses its force as an indication of the combination of hereditary characters of the wolf with those of the dog.

In connexion with the question of the dogs' and wolves' capacity to learn and unlearn barking may be mentioned the *heterogeneity* of our hybrids according to their "psychic" characters. This is connected with their belonging to different types of higher nervous activity.

Some of the hybrids are easily excitable, others markedly lethargic, which is readily evident, even in a single litter where definite segregation may be observed. Thus, male no. 12  $F_2$  and female no. 26  $F_2$  are very lethargic, while male no. 20 is very aggressive and easily excitable and male no. 42 a rather inert and torpid dog.

Certain data may also be cited on the susceptibility of wolf-dogs to *training*. Like ordinary domestic dogs all our  $F_1$  and  $F_2$  hybrids may be trained and the majority were so. Some of them were trained for sledge and ski teams and proved to be good service dogs.

Moreover, the experiments we carried out at the Moscow Zoopark demonstrated the possibility of training and taming pure wolves born in captivity or caught wild (both as puppies or as adult animals). Our collaborators taught old wolves to obey the command "run", "here", "slowly", "quietly", "back", "don't" (No), "hop", etc.

Here we may mention a peculiar "psychical defect" in a number of  $F_1$  and  $F_2$  hybrids in comparison with dogs and wolves. It shows itself in a marked passive-defensive reaction. Sherman (1930) also mentions a certain "psychological" defectiveness of wolf-dogs.

In conclusion we may refer to another peculiarity in the behaviour of wolf-dogs. The custom of the domestic dog, noticed by Linnaeus, of lifting its hindleg during urination was also observed in our hybrids. The only exception was the male no. 7a, which carried out this operation in a sitting position, almost like a female. This custom is observed in our hybrids as well as in dogs beginning with the age of 4-6 months. (In dogs this custom is normally manifested at the age of 4-6, but sometimes, though rarely, after 12 months.)

## SUMMARY

The wolf and the dog can be readily crossed and the resulting hybrids are fully fertile. Typical Mendelian segregation has been demonstrated for many different characters, notably for hair colour and pattern, eye colour, ear form, size and various skull characters. There is also evidence for segregation in certain physiological peculiarities such as season of rut and nervous disposition. Modificatory influences due to environment may affect some of the craniological characters, the form of the tail and the general external appearance, though in all of these cases the differences are at basis genotypical. The bark of the dog is shown to be a purely modificatory character which may be readily acquired by the wolf. Certain features such as the duration of pregnancy, the blind period in the young, the order of appearance of milk teeth and the moulting phenomena are identical in both wolves and dogs. All of these data taken together serve to emphasize the very close similarity in genetical constitution between the wolf and the dog, and suggest the *possibility* of the origin of the various races of *Canis familiaris* from a single wild species, viz. *C. lupus*.

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## EXPLANATION OF PLATES 19-29

## PLATE 19

- Fig. 1.  $F_1$  wolf-dog hybrid (male), "Skukum", zonar-grey.  
 Fig. 2.  $F_1$  wolf-dog hybrid (female), "Mamzel", zonar-grey.  
 Fig. 3.  $F_1$  wolf-dog hybrid (male), "Mak", black.

## PLATE 20

- Fig. 4.  $F_2$  wolf-dogs, offspring of ♂ "Mak" × ♀ "Zizi".  
 Fig. 5.  $F_2$  wolf-dogs (of one litter).  
 Fig. 6. Greyish white markings on  $F_2$  wolf-dog, male, no. 20.

## PLATE 21

- Fig. 7.  $F_2$  brown-fulvous wolf-dog, male, no. 42.  
 Fig. 8. A big fulvous wolf-dog "Boorij" with whitish blue iris (left) and a zonar-grey one "Malishka" (right).  
 Fig. 9.  $F_2$  wolf-dogs (from left to right): enormous fulvous male no. 42, middle-sized black and tan male no. 33, and small zonar-grey female no. 43.

## PLATE 22

- Fig. 10. A zonar wolf-grey German sheep-dog, female, "Aza".  
 Fig. 11. A black, self-coloured German sheep-dog, male, "Astor".

## PLATE 23

- Fig. 12. Illustrating difference in size of adult  $F_2$  wolf-dogs.  
 Fig. 13.  $F_2$  wolf-dog, male, no. 3, "Els".  
 Fig. 14.  $F_2$  wolf-dog, no. 21.

## PLATE 24

- Fig. 15. Skulls of wolves. "Acute" angle of the pr. zyg. max.  
 Fig. 16. Skulls of German sheep-dogs with "open" angle of the pr. zyg. max.

## PLATE 25

- Fig. 17. Skulls of the  $F_1$  wolf-dogs nos. 4 and 3 showing "intermediate" (above) and "obtuse" (below) angles of the pr. zyg. max.  
 Fig. 18. Skulls of the  $F_1$  wolf-dogs nos. 1 and 2 showing "intermediate" angle of the pr. zyg. max.

## PLATE 26

- Fig. 19. Skulls of  $F_2$  progeny from a cross between  $F_1$  male "Mak" and female "Zizi".  
 Fig. 20. Skulls of  $F_2$  progeny from a cross between  $F_1$  male "Skukum" and female "Mamzel".

## PLATE 27

- Fig. 21. Skulls of wolves, dogs and  $F_1$  wolf-dogs (from below).

## PLATE 28

Fig. 22. Four types of the shape and the size of the bullae tympani in  $F_2$  wolf-dogs convex, slightly rumpled, rumpled and flattened out (from left to right).

## PLATE 29

Fig. 23. Wolf skulls, broad in cheek-bones (right) and narrow in cheek-bones (left).

Fig. 24. German sheep-dog skulls showing small cheek-bone breadth (compare Pl. 29, fig. 23).

Fig. 25. Skulls of  $F_1$  wolf-dogs with "intermediate" cheek-bone breadth.

Fig. 26. Skulls of  $F_1$  wolf-dogs with "intermediate" cheek-bone breadth.



Fig. 1.



Fig. 3.

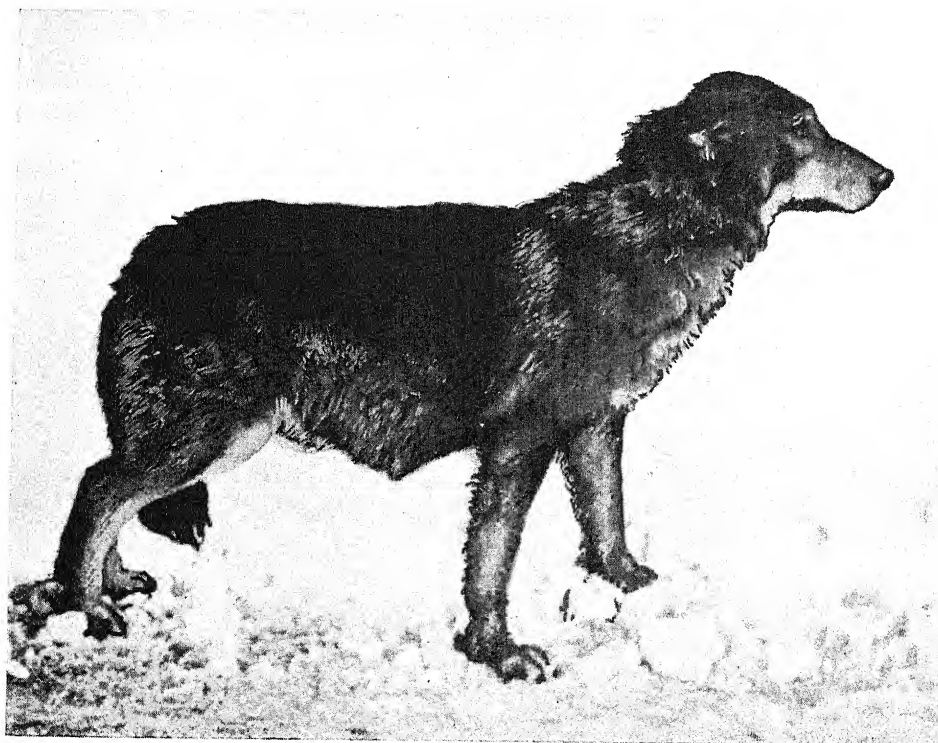


Fig. 2.





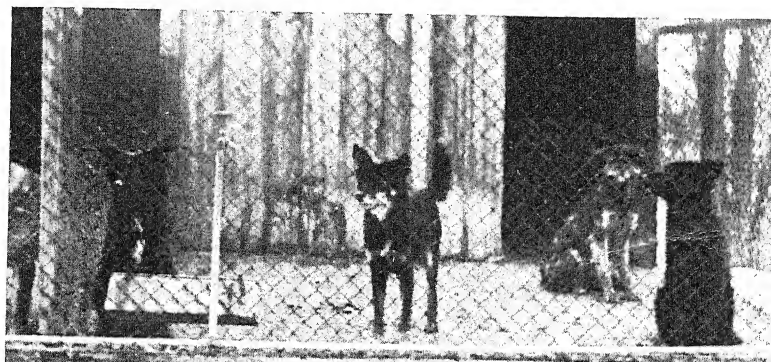


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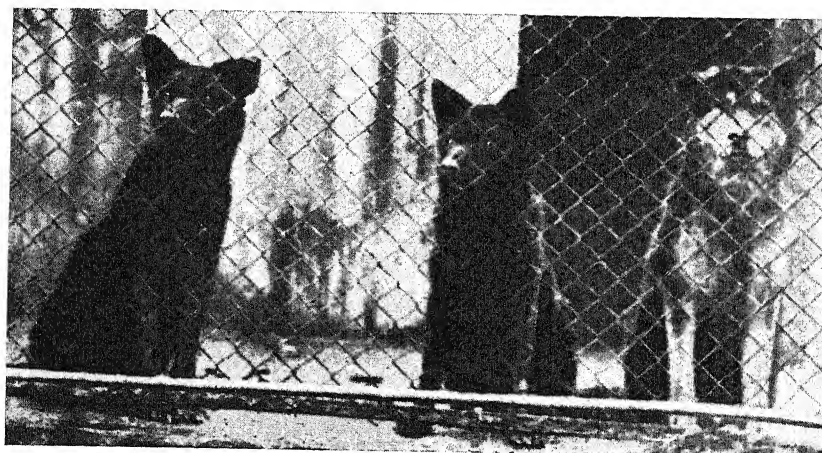


Fig. 5.

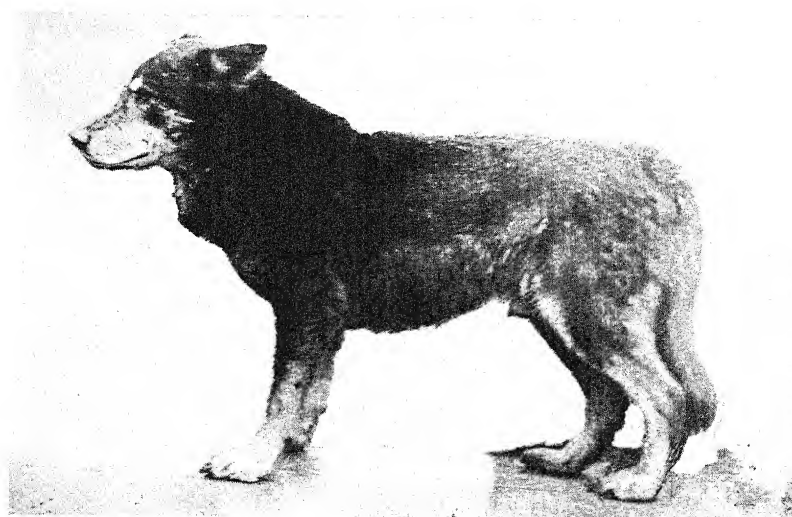


Fig. 6.



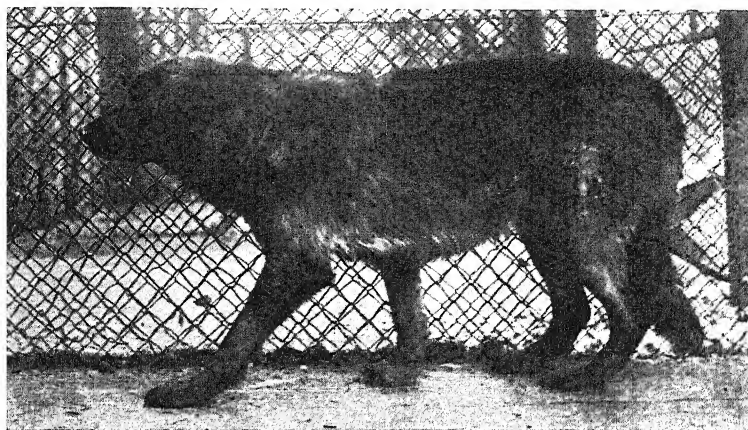


Fig. 7.



Fig. 8.

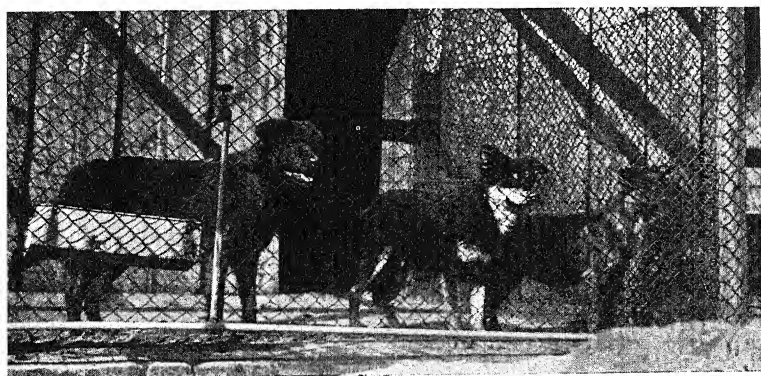


Fig. 9.



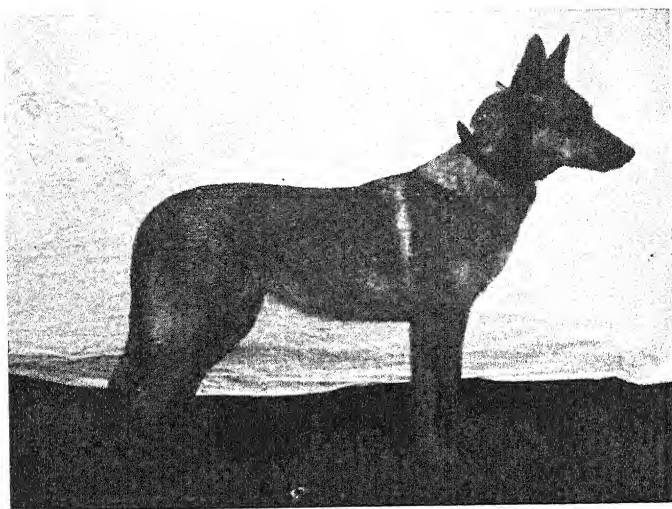


Fig. 10.



Fig. 11.





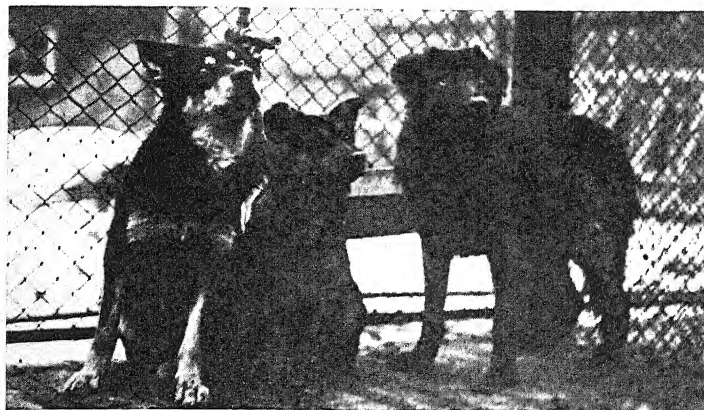


Fig. 12.



Fig. 13.



Fig. 14.





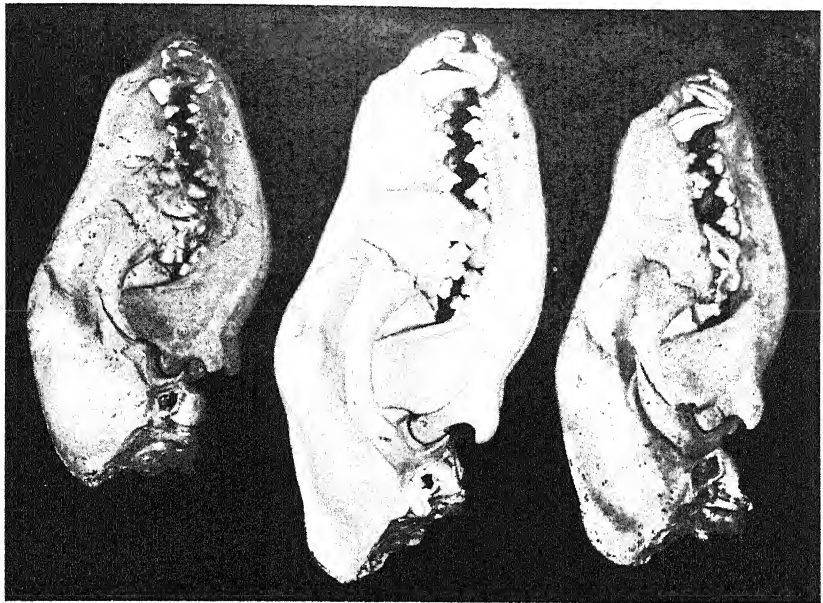


Fig. 16.

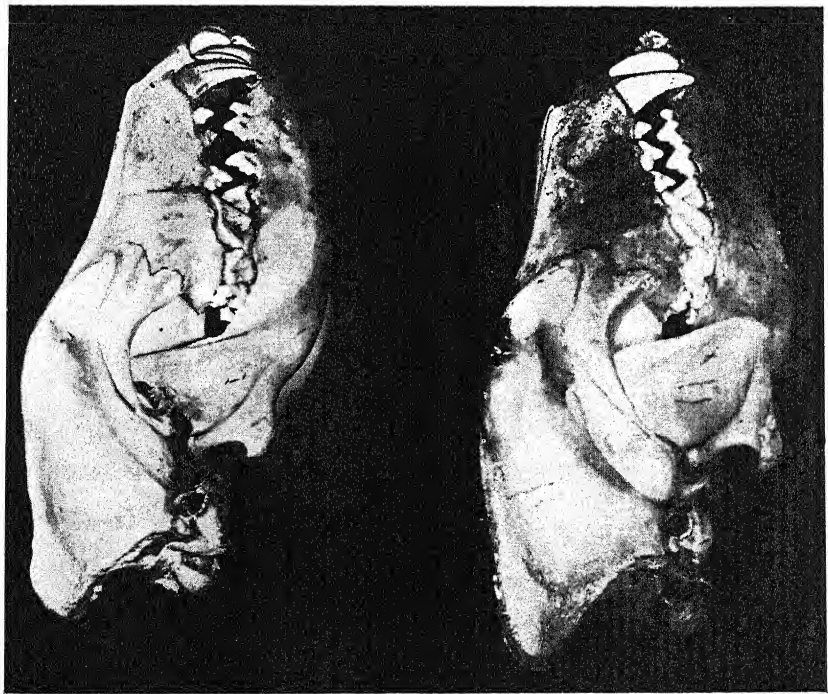


Fig. 15.



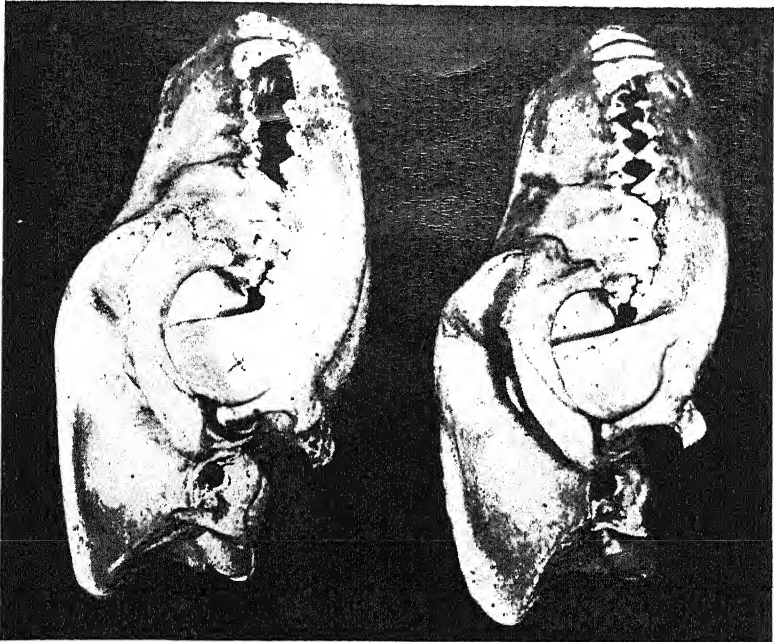


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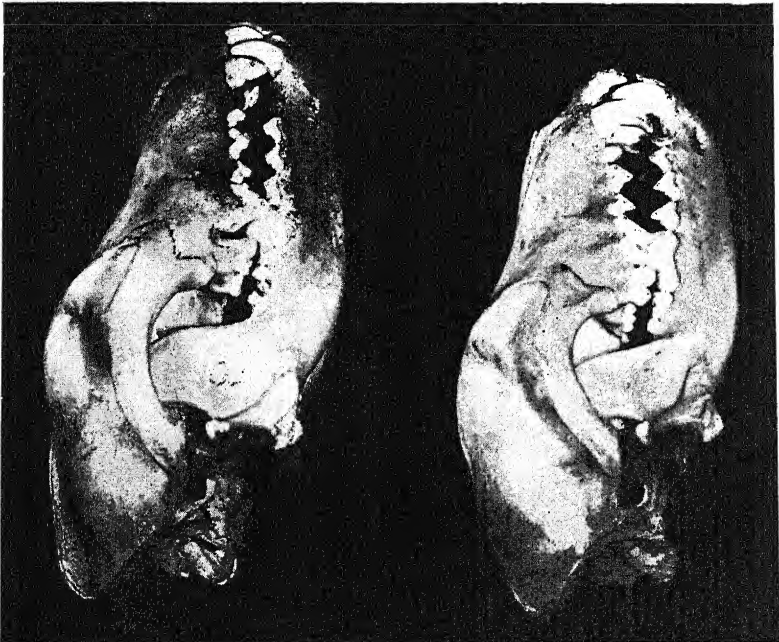


Fig. 17.

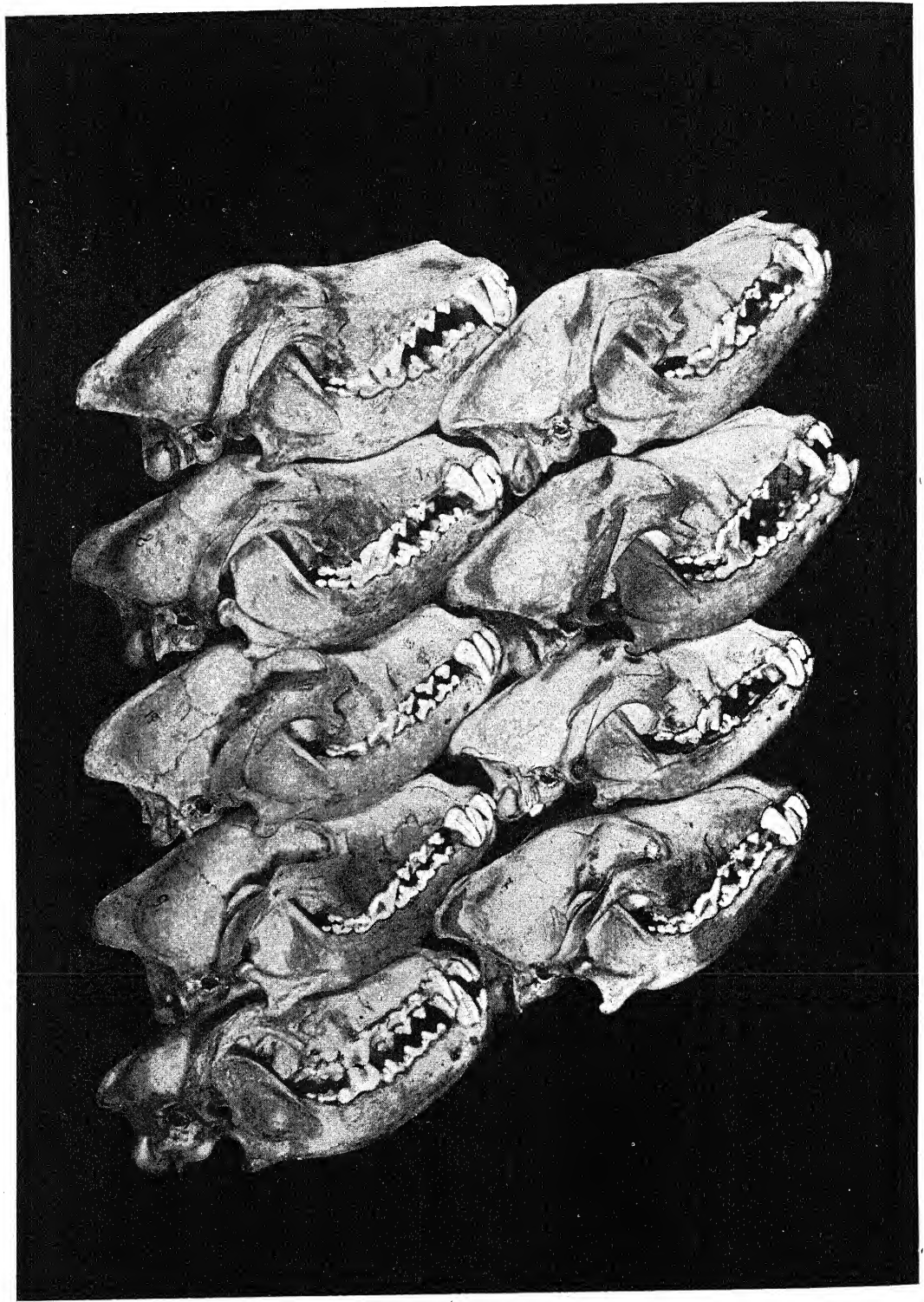


Fig. 19.



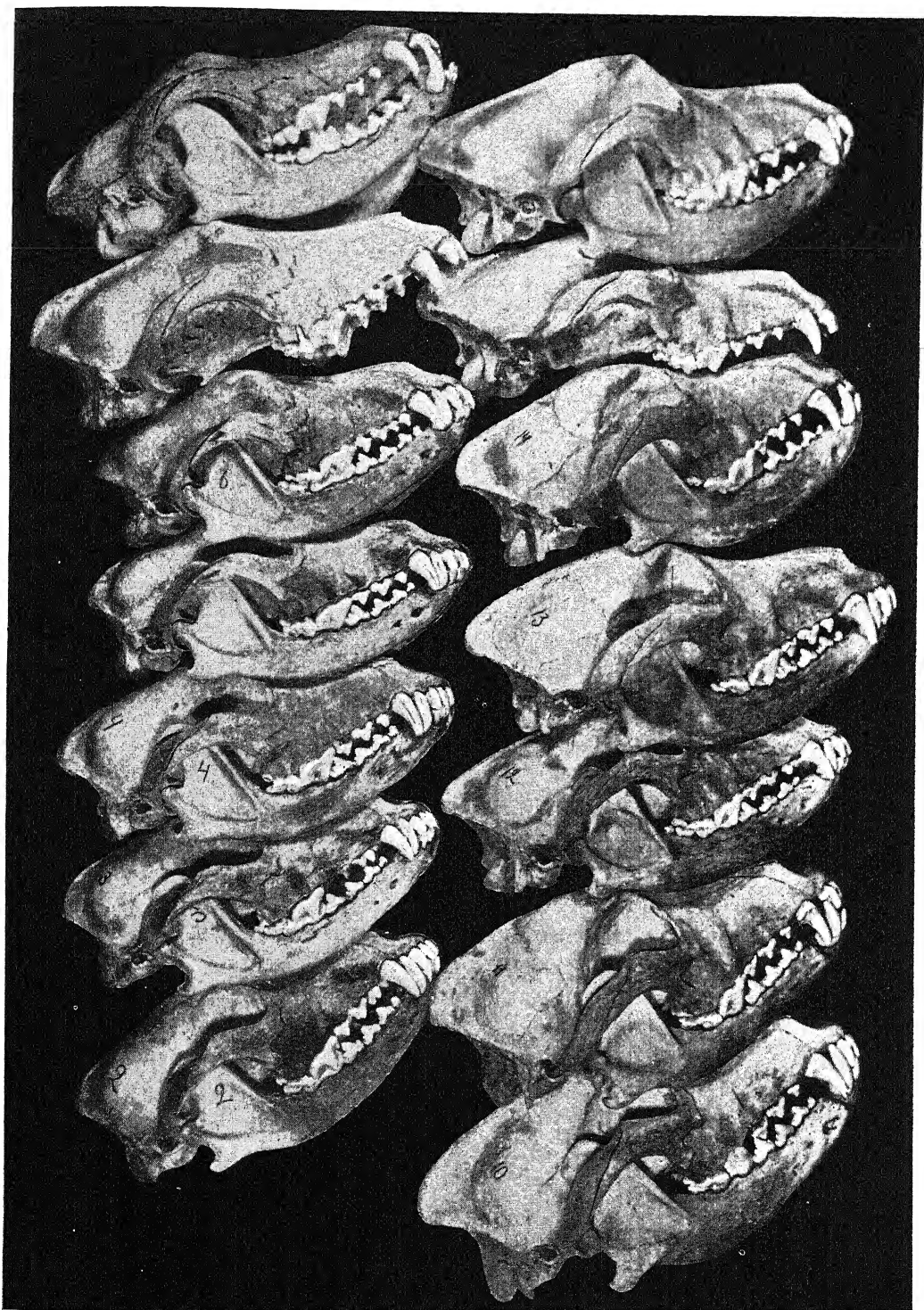


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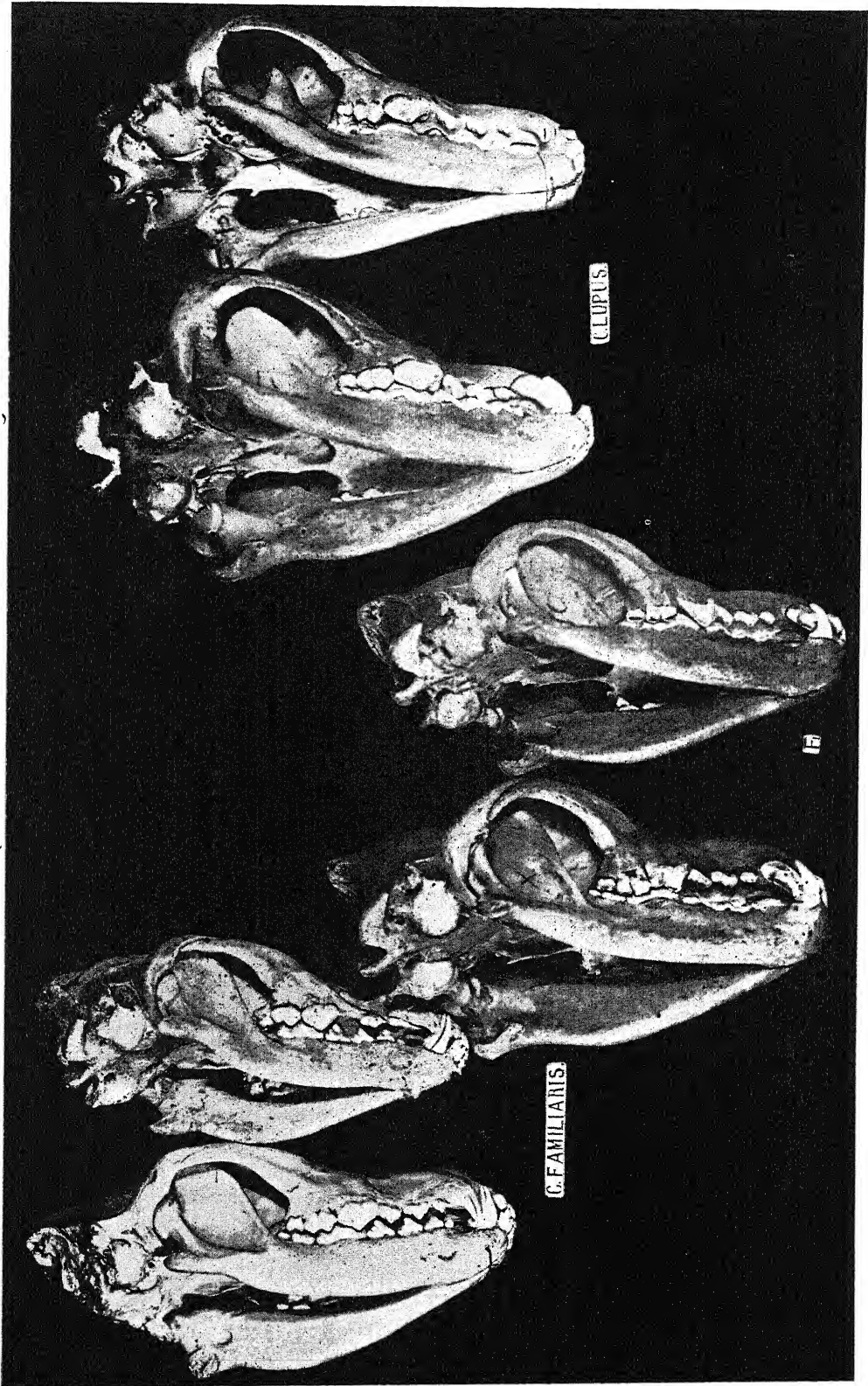


Fig. 21.





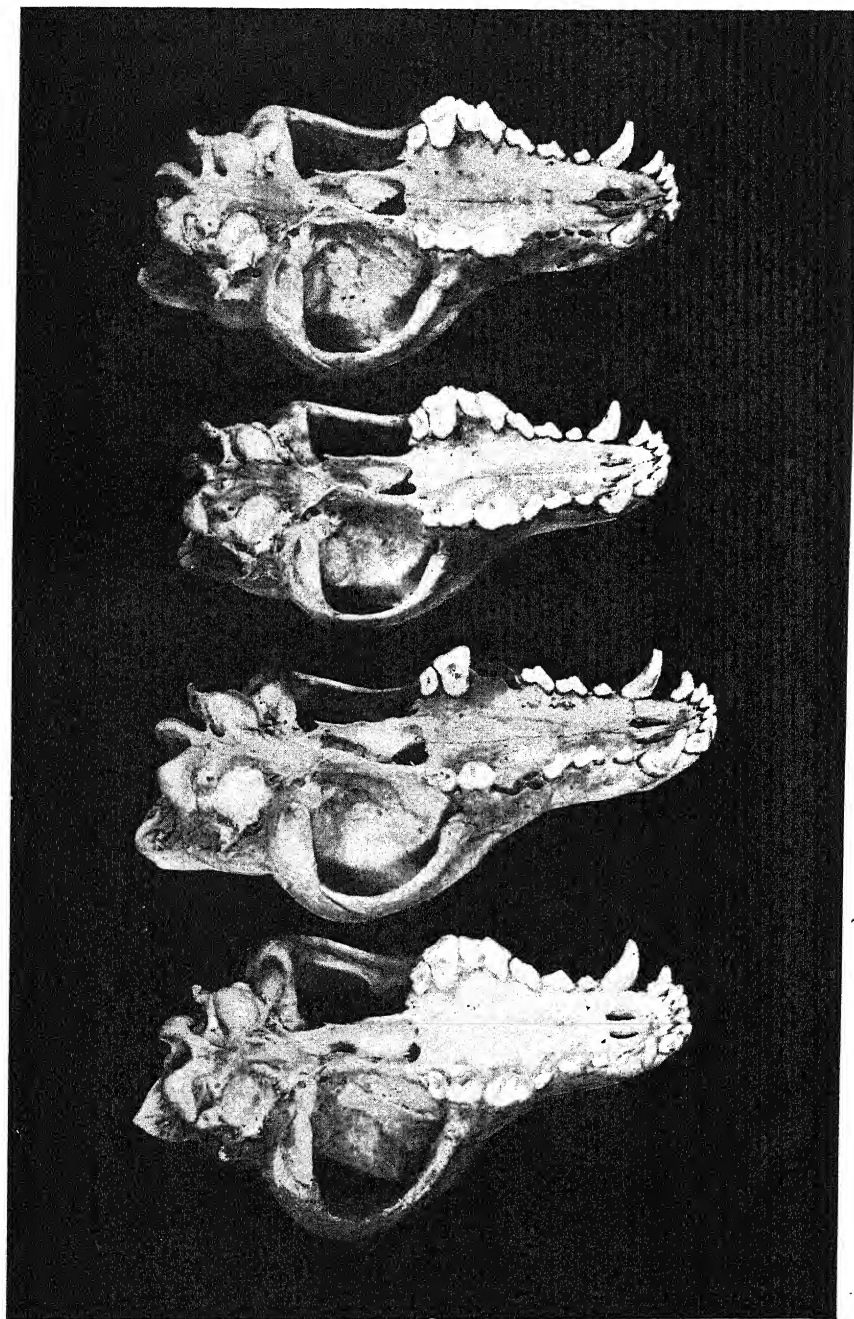


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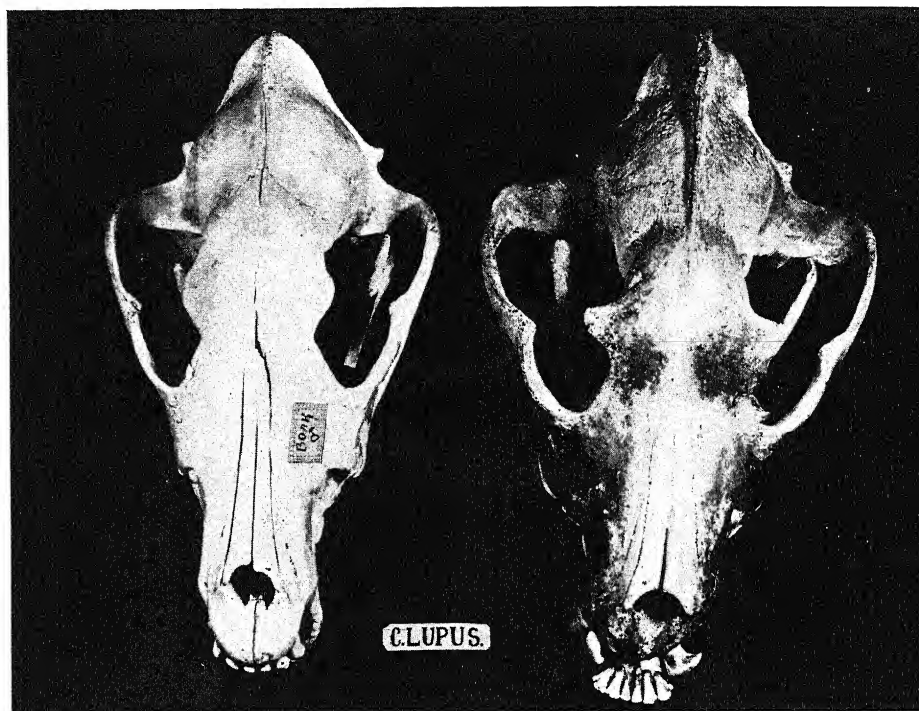


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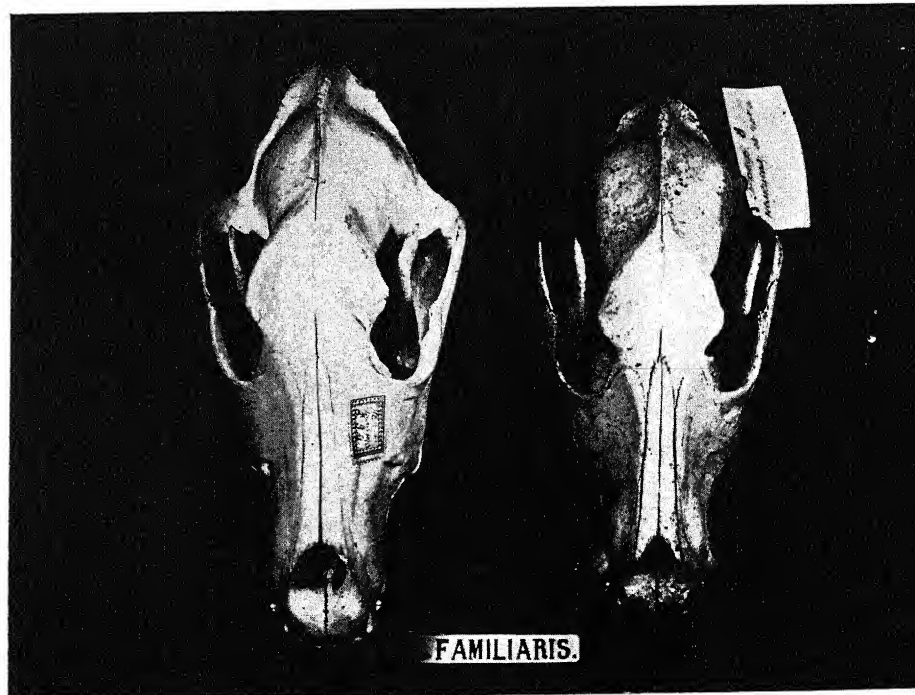


Fig. 24.



Fig. 25.

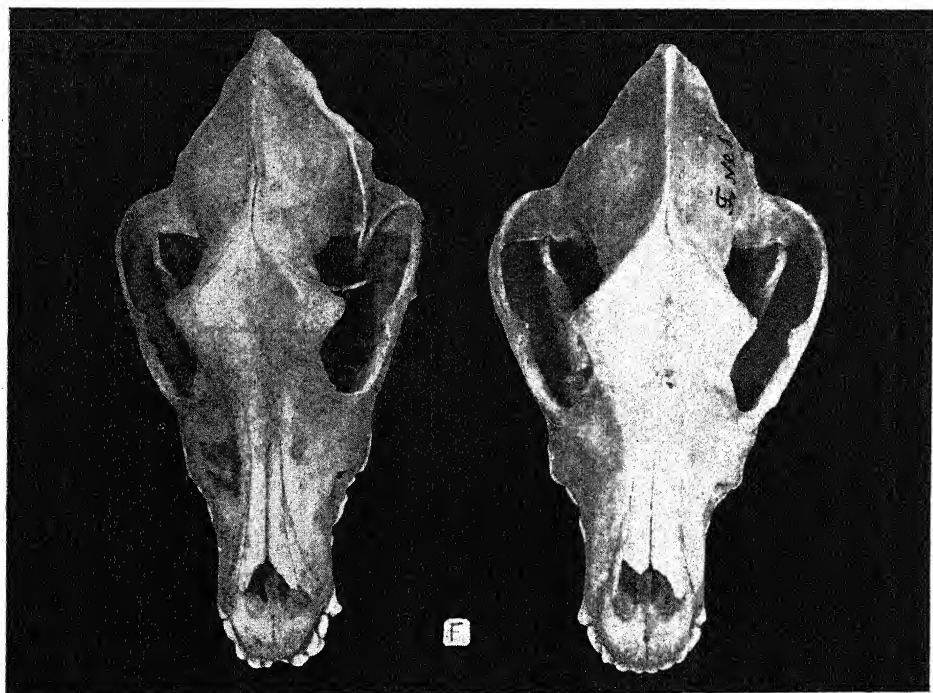


Fig. 26.